Effects of Copper and Histidine on Oxidative Modification of Low Density Lipoprotein and Its Subsequent Binding to Collagen

Norman Kalant, Suzanne McCormick, and Michael A. Parniak

It was previously shown that serum low density lipoprotein (LDL) binds to type I collagen gels and that the binding is increased after modification by cultured endothelial cells. It is now demonstrated that when LDL is incubated with cells cultured in Dulbecco's modified minimal essential medium (DMEM), the subsequent binding of LDL to collagen is considerably less than after incubation with endothelial cells cultured in Ham's F-12 medium (F12). To determine the reason for this difference, collagen gels were made with saline containing ingredients of DMEM individually or in groups, and binding of LDL to such gels was measured. Modification of LDL, manifested by a high level of binding to the collagen, by lipid peroxidation (production of thiobarbituric acid–reactive substances), and by increased electrophoretic mobility occurred on exposure to collagen gels made in saline; these changes were almost completely inhibited by the addition of histidine at a concentration equal to that in DMEM. They were also inhibited by butylated hydroxytoluene, desferrioxamine, and EDTA; penicillamine and hydroxyl-radical scavengers inhibited collagen binding but did not inhibit lipid peroxidation or the increase in electrophoretic mobility. Nominally, DMEM contains 270 μM histidine but no copper, whereas F12 contains 135 μM histidine and 10 nM copper; addition of copper (as much as 5 μM) to DMEM or of histidine (as much as 2.16 mM) to F12 did not overcome the differences between the media in supporting LDL oxidation by endothelial cells. It is concluded that 1) modification of LDL in saline, which results in increased binding to collagen, is dependent on free radicals generated in the presence of trace amounts of copper; 2) histidine is a potent inhibitor of this process; and 3) although the relative inability of DMEM in comparison to F12 to support such modification of LDL by endothelial cells may be partly due to its lower content of copper and its higher content of histidine, other differences in composition are important factors. It is hypothesized that copper ion binds to histidine residues of the LDL protein and, while bound, catalyzes production of hydroxyl radicals, which degrade the protein, producing or uncovering collagen-binding sites. (Arteriosclerosis and Thrombosis 1991;11:1322–1329)
ble residues in the apolipoprotein itself. The goal of the present work was to examine some of the factors that might influence oxidation of the apolipoprotein and thus, its binding to collagen.

Earlier studies have demonstrated that oxidation of LDL lipids by ECs occurs when the cells are cultured in Ham's media F-10 and F-12 (F12) but not in Dulbecco's modified minimal essential medium (DMEM). It also occurs in cell-free phosphate-buffered saline (PBS) containing added Cu**; this has led to the view that Cu** plays a critical role in catalyzing lipid oxidation. We have examined the culture media and the role of Cu** in regard to alterations in LDL, as reflected by binding of the protein to collagen; electrophoretic mobility was monitored as an independent indicator of change in LDL.

**Methods**

**Cell Culture**

Endothelial cells were obtained from porcine aortas by the procedure of Gimbrone et al and maintained in culture in DMEM (obtained from Flow Laboratories, Mississauga, Canada) supplemented with 1 mM nonessential amino acids (GIBCO Canada, Burlington, Ontario), NaHCO₃ (2.25 g/l) and 10% (vol/vol) defined supplemented calf serum (HyClone Laboratories, Logan, Utah) in dishes coated with human fibronectin. They were passaged at confluence once weekly by exposure to 0.05% trypsin/0.02% Na₂EDTA in Earle's salt solution (GIBCO), followed by a wash in serum-containing medium. They were redistributed at a 1:20 split ratio and given fresh medium every 2 days.

To prepare cultures for incubation with LDL, cells between the second and 15th passages were seeded in 60-mm tissue-culture dishes 1 week before use and grown to confluence under conditions described above for stock cultures.

**Preparation of Low Density Lipoprotein and Iodine-125-Labeled Low Density Lipoprotein**

Normolipemic serum obtained from the hospital diagnostic laboratories was pooled, and after addition of Na₂EDTA (final concentration, 0.01%), LDL was isolated by precipitation with heparin and MgCl₂. Very low density lipoprotein that coprecipitated was removed by ultracentrifugation in NaCl at a density of 1.006 g/ml. The purified LDL was dialyzed against the NaCl/EDTA buffer, more than 98% of the radioactivity was precipitable by cold 10% (wt/vol) trichloroacetic acid (TCA), and less than 3% was extractable in chloroform/methanol (2:1, vol/vol). The final specific activities ranged from 90 to 150 cpm/ng LDL protein. All LDL preparations were sterilized by filtration through a 0.22-μm membrane, stored in the dark at 4°C, and used within 3 weeks.

**Incubation of Low Density Lipoprotein With Cells**

Confluent cell cultures in 60-mm dishes were rinsed twice with Dulbecco's PBS (GIBCO). They were incubated with 2 ml serum-free F12 (Flow) containing 200 μg ¹²⁵I-LDL protein/ml. Dishes without cells were incubated in the same fashion to provide what will be referred to as cell-free modified LDL. After 24 hours, the conditioned medium was collected under sterile conditions and centrifuged for 10 minutes at 1,000g to remove cell debris. An aliquot was taken to measure the amount of radioactivity precipitable in cold 10% TCA; this was routinely in the range 92–96%. The conditioned medium was diluted in DMEM to the desired concentration of ¹²⁵I-LDL protein to be added to collagen gels prepared as described below.

For purposes of electrophoresis, unlabeled LDL (200 μg protein/ml) was incubated for 24 hours with or without cells, and the conditioned medium was concentrated to approximately 2 mg LDL protein/ml in a dialysis bag (molecular weight [MW] retention limit of 3,500) immersed in a thick slurry of polyethylene glycol (MW 8,000; BDH Chemicals, Toronto, Canada) in water. Electrophoresis was performed on agarose gel films prepared from GelBond film and Seakem ME agarose (both from FMC Bio-products, Rockland, Me.) in barbital buffer, pH 8.6, at 200 V for 45 minutes. Of the concentrated medium containing modified LDL, 20 μl was applied to the agarose gel. Native (unincubated) LDL was diluted to the same concentration and applied as a control in each electrophoretic run. Dried gels were stained with Sudan black B dye according to Johansson. The distance traveled by each sample was measured in millimeters from the origin to the front of the band. Relative mobility was expressed as a ratio of the migration distance of sample to that of native LDL.

**Preparation of Collagen Gels and Measurement of Binding of ¹²⁵I-Low Density Lipoprotein**

Type I collagen was extracted from fresh rat tail tendon with dilute acetic acid under sterile conditions. The final collagen concentration of individual preparations, determined by dry weight, was 2–3 mg/ml. To prepare collagen gels, the collagen solution was mixed with the appropriate concentrated medium (DMEM, NaCl in water, or F12, as described in each experiment) in volumes adjusted to provide a final concentration of 1 mg/ml collagen in a medium of 1× concentration or 0.15 M NaCl (saline); in the case of saline, NaHCO₃ was added to provide the same concentration as in DMEM. Aliquots (1 ml) of this solution were pipetted into 35-mm culture dishes and incubated at 37°C for 1 hour to allow the collagen to gel completely. The gels
were covered with 1 ml medium containing 50 μg 125I-LDL protein and incubated for 48 hours.

The overlying medium was aspirated, and the gel was rimmed with a spatula to release it from the plastic substrate. It was transferred to a Whatman No. 541 filter paper on a metal support screen fitted with a metal cylinder to facilitate washing over suction. The collagen gel was washed 12 times with 22-ml volumes of PBS, transferred to a test tube, and dissolved in 3 ml 1N NaOH. Aliquots were taken for measurement of 125I and for protein measurement.

All reagents were prepared with deionized water having a resistivity greater than 2MΩ; in several comparisons identical results were obtained with high-performance liquid chromatography-grade water. The details of all procedures have been described previously.1

**Analytical Methods**

Protein was measured by the procedure of Lowry et al; thiobarbituric acid–reactive substances were measured by the method of Schuh et al, with malondialdehyde bis(dimethylacetal) as a standard.

**Results**

LDL was incubated in either DMEM or F12 in the absence or presence of ECs and then tested for binding to collagen gels made in DMEM and for alterations in electrophoretic mobility. Incubation with ECs in F12 resulted in marked increases both in the ability of LDL to bind to collagen and in electrophoretic mobility (Table 1). By contrast, incubation of LDL with ECs in DMEM had virtually no effect on these parameters. Thus, DMEM permits the binding to collagen of LDL previously modified by exposure to ECs in F12 and then assayed for binding to saline occurred in F12 and in saline; this result was obtained even in the absence of ECs. Because NaCl, the only component of saline, is also contained in DMEM, the low level of binding of native LDL to DMEM gels must have been due to some constituent that is inhibitory; because F12 shares most of the components of DMEM, this inhibitory effect is probably due to a quantitative difference in the composition of the media.

To determine the factor(s) responsible for this inhibition, various components of DMEM were added to saline at the same concentration as in DMEM, and these supplemented saline solutions were used to prepare collagen gels for assay of native LDL binding. As shown in Table 3, the DMEM–amino acid mixture inhibited binding completely, and the DMEM–vitamin mixture inhibited binding by about 65%; the salt mixture and glucose had no effect.

The inhibitory amino acids were subsequently determined to be histidine and to a lesser extent tyrosine (Table 3). The inhibition of binding to saline collagen gels and of the increase in electrophoretic mobility of LDL was dependent on the concentration of histidine and tyrosine (Figure 1). Analogues of histidine were in general less effective than histidine itself (Table 4).

To determine if the action of histidine was on the oxidation of LDL or the subsequent binding of the protein to collagen, LDL was oxidized by exposure to ECs in F12 and then assayed for binding in the presence of BHT (to inhibit further oxidation by free radicals), histidine, or both BHT and histidine. The results (Table 5) show that BHT did partially inhibit binding, indicating that some further oxidation occurred in the binding gel in the absence of BHT but that histidine inhibited binding by 20% in the presence and absence of BHT; thus histidine, in addition to its inhibitory effect on oxidation, has a direct although a smaller inhibitory effect on binding. The
TABLE 3. Effect of Dulbecco's Modified Minimal Essential Medium Components on Binding of Native Low Density Lipoprotein to Collagen Gel Prepared in Dulbecco's Modified Minimal Essential Medium, Saline, or Ham's F-12 Medium

<table>
<thead>
<tr>
<th>Gel</th>
<th>Component added</th>
<th>Binding (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM</td>
<td>...</td>
<td>0.36±0.02</td>
</tr>
<tr>
<td>Saline</td>
<td>...</td>
<td>11.12±0.28</td>
</tr>
<tr>
<td></td>
<td>Salts</td>
<td>11.69±0.57</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>9.22±0.18</td>
</tr>
<tr>
<td></td>
<td>Salts+glucose</td>
<td>12.26±0.36</td>
</tr>
<tr>
<td></td>
<td>Salts+glucose+amino acids</td>
<td>0.57±0.04</td>
</tr>
<tr>
<td>F12</td>
<td>Histidine (270 μM)</td>
<td>0.45±0.04</td>
</tr>
<tr>
<td></td>
<td>Tyrosine (397 μM)</td>
<td>2.99±0.13</td>
</tr>
<tr>
<td></td>
<td>Vitamins</td>
<td>3.71±0.15</td>
</tr>
<tr>
<td></td>
<td>Histidine (135 μM)</td>
<td>10.69±1.04</td>
</tr>
</tbody>
</table>

Native iodine-125-labeled low density lipoprotein (LDL) was incubated with collagen gels prepared in saline alone or containing components of Dulbecco's modified minimal essential medium (DMEM), and binding to collagen was measured. Extent of binding is compared with collagen binding in DMEM and in Ham's F-12 medium (F12) with and without added histidine. Additions to saline gels gave final concentrations equivalent to those of DMEM. Because histidine concentration of F12 is 135 μM, addition of a further 135 μM provided the same concentration as in DMEM. Results are mean±SD from two or more experiments, each in quadruplicate.

Addition of histidine to F12 to provide the same concentration as in DMEM had no inhibitory effect; only at a concentration about eightfold higher than that in DMEM was EC-mediated oxidation blocked.

FIGURE 1. Concentration–response curve showing effect of histidine and tyrosine (both in μM) on low density lipoprotein (LDL) binding. Collagen gels were prepared in saline; 1.0 ml saline containing 50 μg iodine-125–labeled LDL and either histidine or tyrosine was added to the gel. Final concentrations of added amino acid in the combined fluid volume (1 ml gel fluid + 1 ml overlying fluid) is shown on the x axis. After incubation (48 hours), measurements were made of LDL bound to the gel (expressed as percent of binding in absence of amino acid) and of LDL relative electrophoretic mobility (for histidine gels only). Relative mobility of native LDL was set to 1. Binding (●) and electrophoretic mobility (×) in the presence of histidine; binding (○) in the presence of tyrosine.

TABLE 4. Inhibition of Low Density Lipoprotein Binding to Collagen Gel by Histidine Analogues (135 μM)

<table>
<thead>
<tr>
<th>Histidine analogue</th>
<th>Binding (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Histidine</td>
<td>4.3</td>
</tr>
<tr>
<td>D-Histidine</td>
<td>4.8</td>
</tr>
<tr>
<td>Histidinol</td>
<td>37.6</td>
</tr>
<tr>
<td>Histidine methyl ester</td>
<td>4.6</td>
</tr>
<tr>
<td>Histidyl leucine</td>
<td>7.3</td>
</tr>
<tr>
<td>Histamine</td>
<td>62.5</td>
</tr>
<tr>
<td>Acetylhistidine</td>
<td>76</td>
</tr>
<tr>
<td>2-Thierylanline</td>
<td>73</td>
</tr>
<tr>
<td>3-Thierylanline</td>
<td>75</td>
</tr>
<tr>
<td>2-Thieryglycine</td>
<td>85</td>
</tr>
<tr>
<td>3-Thieryglycine</td>
<td>87</td>
</tr>
<tr>
<td>Imidazole</td>
<td>107</td>
</tr>
</tbody>
</table>

Collagen gels were prepared in saline. Values for binding are based on a minimum of five experiments, each in quadruplicate.

The histidine-dependent inhibition of the binding of LDL to collagen is unlikely to be due to interaction as determined by subsequent binding to a DMEM–collagen gel (Figure 2).

The histidine-dependent inhibition of the binding of LDL to collagen is unlikely to be due to interaction

TABLE 5. Effect of Histidine and Butylated Hydroxytoluene on Binding of Endothelial Cell-Modified Low Density Lipoprotein to Collagen Gel

<table>
<thead>
<tr>
<th>Addition to binding gel</th>
<th>Binding (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.53±0.39</td>
</tr>
<tr>
<td>BHT</td>
<td>6.43±0.42</td>
</tr>
<tr>
<td>Histidine</td>
<td>7.67±0.45</td>
</tr>
<tr>
<td>BHT+histidine</td>
<td>5.03±0.32</td>
</tr>
</tbody>
</table>

Low density lipoprotein (LDL) that had been incubated with endothelial cells in Ham's F-12 medium was applied to collagen gels in saline containing 20 μM butylated hydroxytoluene (BHT), 135 μM histidine, or both. Results (mean±SD) are based on two experiments, each in quadruplicate.

FIGURE 2. Concentration–response curve showing effect of histidine concentration (mM) on oxidation of low density lipoprotein (LDL) by endothelial cells. Iodine-125–labeled LDL was incubated with cells in Ham's F-12 medium to which histidine was added to provide concentrations shown; binding of LDL to collagen gels made with Dulbecco's modified minimal essential medium was then measured.
Role of Metals in Promoting the Binding of Low Density Lipoprotein to Saline-Collagen Gels

Transition metal ion chelators such as EDTA are required to minimize oxidation during the isolation of LDL. To determine whether the low extent of LDL oxidation by ECs in DMEM was due to a lack of transition metal ions in this medium, native LDL was incubated with ECs in DMEM, to which had been added CuSO4, FeSO4, and ZnSO4, to final concentrations double those in F12 (because the concentration of histidine in DMEM is twice that in F12). The modified DMEM required higher metal levels to maintain the same metal to histidine ratio. The binding of LDL to a collagen gel in unmodified DMEM was not significantly affected. When DMEM was supplemented with Cu2+ to 5 μM, the subsequent binding to collagen and electrophoretic mobility of LDL were increased but not to the extent noted with F12 (Table 7). Although no metal salts were added to the saline gels, the ubiquitous distribution of Cu2+ and Fe3+ raised the possibility that trace amounts of these metal ions in the saline solution might catalyze LDL oxidation, thereby increasing LDL binding to saline–collagen gels. This binding was therefore measured in the presence of several metal chelators and of oxygen-radical scavengers. Histidine, EDTA, and desferrioxamine abolished the binding, the increase in electrophoretic mobility of LDL, and the production of thiobarbituric acid–reactive substances (Figure 3 and Table 8). By contrast, penicillamine had no effect on either the production of thiobarbituric acid–reactive substances or the increase in relative electrophoretic mobility of LDL, despite providing a significant inhibition of the binding of LDL to collagen. BHT, a "general" free-radical scavenger, also inhibited all three parameters, whereas n-butanol, formate, thiourea, azide, and tris(hydroxymethyl)amino- methane, which are water-soluble hydroxyl-radical scavengers, inhibited only the binding.

Discussion

It is well known that LDL is readily oxidized and that during isolation and manipulation, it must be...
Low density lipoprotein (LDL) is oxidized by ECs and other cell types; the extent of oxidation is dependent on the cell culture medium, occurring to a much greater extent in F10 and F12, which contain Cu²⁺, than in DMEM, which does not; the observation that oxidation is produced in the absence of cells by incubation of LDL in PBS containing 5μM Cu²⁺ lent support to the concept that transition metals play an important role in the oxidative process.

Morel et al demonstrated that LDL underwent oxidation with production of thiobarbituric acid-reactive substances and increased electrophoretic mobility on incubation in isotonic saline even without the addition of Cu²⁺. The present results with a saline-collagen gel confirm the latter observation and, in addition, show the associated increase in ability of LDL to bind to collagen. Such oxidation is inhibited by the presence of BHT, a free-radical scavenger, or of desferrioxamine, penicillamine, or EDTA, which chelate heavy metals, including copper. Thus, it is possible that metal ions need be present in only minute amounts (presumably as a contaminant in the saline) to exert a catalytic effect on oxygen-radical formation and consequently on LDL oxidation.

The addition of Cu²⁺ to DMEM to provide a concentration double that of F12 did not lead to significant protein oxidation; even a concentration of 5μM, approximately 500-fold higher than in F12, permitted only a modest level of oxidation. Thus, the presence of Cu²⁺ in F12 (nominally at a concentration of 10 nM) cannot be responsible for the difference between F12 and DMEM in the ability to support LDL protein oxidation. This result is analogous to the observation that ECs can oxidize LDL when cultured in medium 199, which by formulation contains no Cu²⁺, but when cultured in DMEM they require the addition of at least 1 μM Cu²⁺ to permit oxidation. A similar situation exists with regard to Fe³⁺ as a factor in promoting oxidation; although there is a correlation between the iron content of several media and their ability to support oxidation of LDL, the extent of oxidation was much lower in DMEM containing 10 μM Fe (Figure 1 of Reference 18) than in F12 containing 2.2 μM (Table 2 of Reference 18). All these findings, together with the observation that extensive oxidation occurs in saline, demonstrate that the inability of DMEM to support oxidation is likely due to the presence of inhibitors rather than to the lack of Cu²⁺. The results also show that histidine is a potent inhibitor of oxidation, as manifested by binding of LDL to collagen, by increased electrophoretic mobility, and by production of thiobarbituric acid-reactive substances and suggest that the twofold higher histidine content of DMEM compared with that of F12 may contribute to the difference between the media in their abilities to support oxidation.
Histidine is a tridentate chelator of metal ions, with a high affinity for copper and a lower but significant affinity for iron (cumulative stability constants of $10^{8.3}$ and $10^9$, respectively). In this respect, histidine is comparable to penicillamine and EDTA (affinity for copper greater than for iron) and different from desferrioxamine (affinity for iron greater than for copper); however, all bind both metals. The results obtained with histidine analogues indicate that an intact imidazole ring as well as the carbonyl function is necessary for maximum inhibition of oxidation; this is in agreement with the known mechanism of metal chelation by histidine. It is therefore probable that histidine inhibition of LDL oxidation is due to removal by chelation of transition metal ions, especially Cu$^{2+}$.

It has been generally assumed that LDL oxidation is initiated by peroxidation of the lipid moiety and that changes in the protein, manifested as increased susceptibility to uptake by macrophages and increased electrophoretic mobility, result from oxidative attack by lipid peroxidation products. The role of Cu$^{2+}$ is assumed to be related to its action as a catalyst of lipid peroxidation, with production of hydroxyl radicals by the Haber-Weiss reaction, and to its ability to form highly reactive complexes with lipid peroxidation products. However, there are two other pathways by which Cu$^{2+}$-generated free radicals may damage the LDL protein: 1) metal ions in the presence of O$_2$ can generate hydroxyl radicals, which can then damage amino acids and sugars, with the production of thiobarbituric acid-reactive substances. Apolipoprotein B, a glycoprotein, may thus be a direct target for attack by hydroxyl ions produced independently of lipid peroxidation. 2) Cu$^{2+}$ may bind not only to free amino acids but also to amino acid residues of proteins, especially histidine. Such bound copper catalysts the production of hydroxyl radicals from O$_2$ or from other substrates, and the free radicals, present at high concentration near the histidine residues, degrade the protein at these sites.

It would be anticipated that chelators would block all actions of Cu$^{2+}$; the results with histidine, EDTA, and desferrioxamine (Table 3) are compatible with this. However, it is also known that transition metals may be active in generating free radicals from O$_2$ even when chelated; the level of such activity is dependent on several characteristics of the metal-chelator complex. The results indicate that the copper–penicillamine complex remains capable of catalyzing lipid peroxidation, with production of thiobarbituric acid–reactive substances and consequent increase in LDL electrophoretic mobility but partially inhibited collagen binding. BHT, an antioxidant by virtue of its ability to act as a proton donor, also blocks oxidation of lipids and apolipoproteins. In contrast, hydroxyl-radical scavengers inhibit the oxidative changes that lead to collagen binding but not to lipid peroxidation or the increase in electrophoretic mobility. Formate, azide, and tris( hydroxymethyl amino methane in fact produced an increase in lipid peroxidation; this may have resulted from the action of secondary radicals produced by interaction with hydroxyl radicals. We therefore propose that binding results from attack on histidine residues in the LDL protein by hydroxyl radicals arising either from lipid peroxidation or in situ from Cu$^{2+}$ bound to the histidine residues. The results with penicillamine suggest that these two sources of hydroxyl radicals are approximately equal in importance. This hypothesis is in keeping with the observation that oxidative degradation is associated with a marked loss of histidine from apolipoprotein B.

Histidine in the medium would compete with the histidine residues of the LDL for binding of Cu$^{2+}$ and thus prevent the oxidation of the apolipoprotein that allows binding to collagen. The ability of a medium to support oxidation of LDL therefore depends in part on the balance between Cu$^{2+}$, which promotes oxidation, and non-LDL chelators such as free histidine, which inhibit the Cu$^{2+}$ effect. DMEM has less copper and more histidine than F12; these differences can partially explain the ability of F12 and the inability of DMEM to permit protein oxidation. However, modifying DMEM to provide the same Cu$^{2+}$ to histidine ratio as in F12 did not lead to an increase in oxidation, whereas histidine added to F12 did not completely inhibit oxidation even at high concentrations; it is therefore probable that other pro-oxidants and antioxidants in DMEM play a significant role. These components may be derived in part from the higher levels of vitamin supplements in DMEM. For example, pyridoxal is present in DMEM at 20 $\mu$M, whereas pyridoxal levels in F12 are only 0.3 $\mu$M. Pyridoxal is a significant chelator of divalent metal cations; the cumulative stability constant for Cu$^{2+}$–pyridoxal complexes can be on the same order as that of Cu$^{2+}$–EDTA at physiological pH.

Histidine appears to exert a direct effect on the binding of LDL to collagen as well as an indirect effect by inhibition of oxidation. When LDL was first oxidized by ECs and then exposed to a collagen gel in the presence of BHT to inhibit further oxidation, histidine inhibited binding to a modest but significant extent. This could have resulted from histidine inhibition of the interaction of LDL with collagen by competition for binding sites; however, we were unable to show specific binding of $^{14}$C-histidine to either collagen or LDL. It is possible that oxidized forms of histidine, present as contaminants in the histidine preparation, acted as competitive inhibitors of binding. We consider this unlikely, given the high level of purity in properly stored commercial preparations of histidine such as that used in the present studies. We are unable to explain the apparently direct inhibitory effect of histidine on the binding of EC-oxidized LDL to collagen.

References


4. Steinbrecher UP, Witztum JL, Parthasarathy S, Steinberg D: Decrease in reactive amino groups during oxidation or endothelial cell modification of LDL. Arteriosclerosis 1987;7:135–143


25. Gutteridge JMC, Wilkins S: Copper salt-dependent hydroxyl radical formation damage to proteins acting as antioxidants. Biochim Biophys Acta 1983;759:38–41


KEY WORDS • low density lipoproteins • binding • antioxidants • collagen • copper • histidine • oxidation
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doi: 10.1161/01.ATV.11.5.1322

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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