Oxidation of high-density lipoprotein HDL₃ leads to exposure of apo-AI and apo-AII epitopes and to formation of aldehyde protein adducts, and influences binding of oxidized low-density lipoprotein to type I and type III collagen in vitro

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INTRODUCTION

High-density lipoproteins (HDLs) are considered to have an important role in the reverse transport of cholesterol from the peripheral tissue to the liver [1–5]. This class of lipoproteins is probably able to counteract the accumulation of low-density lipoprotein (LDL) in the arterial intima, which is a key event in early atherosclerosis [6]. LDL is assumed to become mildly oxidized by endothelial cells when entering the arterial wall and to recruit monocytes, which become resident macrophages [7]. These cells, as well as smooth-muscle cells, are able to generate free radical species, which in turn oxidatively modify LDL severely to a form recognized by scavenger receptors [8,9]. Accumulation of lipids in macrophages leads to an enhanced transformation of these cells to the so-called foam cells, which are typical cells in early atherosclerotic lesions [10]. In contrast, it was shown that HDL added to the medium of cultured lipid-laden foam cells [6] led to a significant efflux of cholesterol from these cells. However, an oxidative environment in the subendothelial space could also cause oxidative modifications of HDL because this lipoprotein was not found to be as well protected against copper-mediated oxidation as LDL. Oxidative modifications might have important consequences for the biological role of HDL in atherosclerosis. Nagano et al. [11] reported that after oxidative modification HDL lost the ability to stimulate the efflux of cholesterol from foam cells. Furthermore, on oxidation the enzymes associated with HDL, such as paraoxonase and platelet-activating factor acetylhydrolase might lose their abilities to protect LDL against oxidation [12] by metabolizing lipid hydroperoxides. Watson et al. [13] proposed that HDL removes oxidized phospholipids from oxidized LDL and platelet-activating factor acetylhydrolase on HDL hydrolyses them into lysocephatidylcholine and fatty acid fragments. Furthermore HDL replenishes mildly oxidized LDL with platelet-activating factor acetylhydrolase, inactivating oxidized phospholipids and transferring them to HDL. An inhibition of lecithin: cholesterol acyltransferase was also obtained after modification of HDL apolipoproteins by aldehydes stemming from lipid peroxidation [14,15]. Although several articles deal with the oxidation of HDL, the information available about the changes in this lipoprotein on a molecular basis is much less than that on LDL. Thus the aim of the present study was to examine the change in the immunological properties of HDL on oxidation, especially the formation of aldehydic neo-epitopes on apolipoproteins AI and AII. In addition, we investigated how the oxidative modification of HDL strongly alters the immunological properties of this lipoprotein and its binding affinity for collagen.

The changes in the immunological properties of apolipoprotein AI (apo-AI) and AII (apo-AII) during the oxidation of the high-density lipoprotein HDL₃ and its influence on the binding of heavily oxidized low-density lipoprotein (LDL) to type I and III collagen were investigated. Oxidation of HDL₃ or Eu⁺⁺⁺-labelled HDL₃ was performed with CuSO₄, varying the time of oxidation. Oxidation of HDL₃ resulted in an increase in lipid hydroperoxides and enhanced the negative charge of this lipoprotein. Immunological studies with a solid-phase sandwich immunoassay revealed a strong increase in binding of Eu⁺⁺⁺-labelled HDL₃ to polyclonal antibodies against apo-AI and apo-AII within the first 4 h of oxidation. Neo-epitopes were also formed by interaction of the apolipoproteins with degradation products from the lipid per-oxidation of polyunsaturated fatty acids, as evidenced by an immunoreaction of oxidized Eu⁺⁺⁺-labelled HDL₃ with antibodies to 4-hydroxynonenal (4-HNE)– and malondialdehyde (MDA)– protein adducts. Western blot analysis of oxidized HDL₃ samples showed, as well as apo-AI and apo-AII bands, larger aggregated apolipoproteins, occurring after 0.5–2.5 h of oxidation. These aggregates were recognized by antibodies to apo-AI and apo-AII as well as by antibodies to 4-HNE- and MDA-protein adducts. Furthermore the original apo-AI monomers and apo-AII dimers decreased during the oxidation. The ability of native and oxidized HDL₃ to prevent the binding of Eu⁺⁺⁺-labelled 24 h-oxidized LDL to collagen on microtitration plates was estimated. Interestingly, 2 h-oxidized HDL₃ competed most with the binding of 24 h-oxidized LDL on collagen type I and type III, followed by native HDL₃. However, 24 h-oxidized HDL₃ was a weaker competitor. Thus oxidative modification of HDL₃ strongly alters the immunological properties of this lipoprotein and its binding affinity for collagen.

1 This paper is in remembrance of Professor Walter Petek to whom we are greatly indebted for his valuable inspiration and early cooperation.

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Behring AG, sheep antiserum against human apolipoprotein AII from Immuno, and the Eu\(^{3+}\)-labelling kit (Delfia\(^{\circledR}\) no. 1240-302) from Wallac Oy. Type I and III collagen were prepared at the Department of Ophthalmology as described previously [16].

**Lipoprotein preparation**

LDL and HDL\(_1\) were isolated from the plasma of normolipaemic, fasting (12–14 h) young male donors with serum lipoprotein(a) levels lower than 1 mg/dl by differential ultracentrifugation with solid KBr to adjust the density. The following fractions were obtained. LDL and very-low-density lipoprotein were separated at a density of 1.063 g/ml (320000 g, 24 h, 10 °C). LDL was separated from very-low-density lipoprotein by adjusting the density to 1.020 g/ml (320000 g, 24 h, 10 °C). HDL\(_2\) was separated from HDL\(_1\) and lipoprotein-deficient serum at a density of 1.125 g/ml (320000 g, 48 h, 10 °C). For further purification of LDL, one more centrifugation was performed at a density of 1.25 g/ml (320000 g, 24 h, 10 °C). Kallikrein inactivator (aprotinin; 1000 units/l; Bayer), Pefabloc (11.2 mg/l; Merck) and EDTA (1 g/l; Merck) were present during all steps of the LDL and HDL\(_1\) preparation. HDL\(_2\) was further purified on heparin-Sepharose CL-6B (Pharmacia Biotech) at 4 °C to remove the apolipoprotein E-containing fraction. Apolipoprotein E-free HDL\(_1\) fractions were collected, concentrated by ultracentrifugation (100000 g, 16 h, 10 °C) and stored at 4 °C. The purity of apolipoprotein E-free HDL\(_1\) was checked by SDS/PAGE. The protein in LDL and HDL\(_1\) was measured by the method of Lowry et al. [17]. The samples were sterile-filtered and stored at 4 °C in the dark until use.

**Labelling of LDL and HDL\(_1\) with Eu\(^{3+}\)**

Eu\(^{3+}\)-labelling of LDL and HDL\(_1\) was performed as described for LDL [16] in 50 mM NaHCO\(_3\), pH 8.3, containing 20 \(\mu\)M 2-carboxy-2,5,7,8-tetramethyl-6-choromol (Trolox; Hofmann LaRoche). The lipoproteins (10 mg/ml total lipoprotein) were incubated with 0.3 mg of Eu\(^{3+}\)-(p-isothiocyanatobenzyl)-diethylentriamine tetra-acid (Delfia\(^{\circledR}\) Eu-labelling kit; Wallac Oy) at 25 °C in the dark for 12 h. Sephadex G-25 chromatography (Pharmacia Biotech) was used for the separation of the labelled lipoprotein fractions from free chelate in 50 mM Tris/HCl, pH 7.8, containing 0.05% NaN\(_3\) and 20 \(\mu\)M Trolox. The labelling yield of the Eu\(^{3+}\)-labelled lipoproteins was between 4 and 8 Eu\(^{3+}\) ions per protein molecule. The fluorescence intensity of Eu\(^{3+}\)-labelled lipoproteins was stable for more than 2 months. The labelled lipoproteins were used within 6 weeks.

**Cu\(^{2+}\)-mediated oxidation of HDL\(_2\), Eu\(^{3+}\)-LDL and Eu\(^{3+}\)-HDL\(_3\)**

Before oxidation, HDL\(_2\), Eu\(^{3+}\)-LDL and Eu\(^{3+}\)-HDL\(_3\) were dialysed against 0.01 M PBS, pH 7.4, which was carefully degassed and then saturated with nitrogen. Cu\(^{2+}\)-mediated oxidation of HDL\(_2\), Eu\(^{3+}\)-HDL\(_3\) (0.75 mg/ml) and Eu\(^{3+}\)-LDL (0.345 mg/ml) was performed at 37 °C with 10 \(\mu\)M CuSO\(_4\). At intervals between 0 and 24 h the reaction was terminated by adding a stop solution to achieve a final concentration of EDTA of 2.7 mM. The samples were saturated with nitrogen and stored at 4 °C in the dark.

The degree of modification of the oxidized lipoproteins was estimated as the relative electrophoretic mobility, i.e. relative to the non-oxidized and unlabelled native LDL or HDL\(_2\), on agarose gels (1 %, w/v) at pH 8.05 with the Lipidophor-system (Immuno AG). Lipid peroxides were estimated by a spectrophotometric assay with iodide colour reagent (Merck) at 365 nm, as developed in this laboratory [18]. To check for possible changes in the fluorescence intensity of Eu\(^{3+}\)-LDL and Eu\(^{3+}\)-HDL\(_3\) occurring during oxidation, the lipoprotein samples were applied to polystyrene microritination plates (Maxisorb; Nunc) in several dilutions with the enhancement solution. The fluorescence counts were measured with the Delfia\(^{\circledR}\) research fluorimeter (Wallac Oy). Oxidation of Eu\(^{3+}\)-LDL or Eu\(^{3+}\)-HDL\(_3\) weakened the fluorescence only slightly.

**Polyclonal antibodies against apo-AI and apo-AII and against HNE- or MDA-protein adducts**

Purification of antisera against apo-AI (Behring AG), and against HNE- and MDA-protein adducts [19,20], all from rabbit, and against apo-AII (Immuno), from sheep, was performed on a DEAE-Sepharose fast flow column (Pharmacia Biotech). After dialysis of the antisera against 50 mM Tris/HCl (pH 8.2)/40 mM NaCl, they were applied to the column and eluted with 50 mM Tris/HCl (pH 8.2)/40 mM NaCl, with monitoring at 280 nm. IgG fractions were collected, dialysed against 50 mM Tris/HCl, pH 8.2, containing NaN\(_3\) at a final concentration of 0.01%, and stored at 4 °C. The purity of all antibodies was checked by SDS/PAGE.

**Solid-phase fluorescence immunassay**

To investigate the formation of oxidation-specific epitopes or the change of native epitopes on apo-AI and apo-AII during the oxidation of HDL\(_3\), an assay was performed as follows: microtitration plates were incubated with a solution of the antibodies (200 \(\mu\)l per well; 5 mg/ml) mentioned above in coating buffer [1.58 g/l Na\(_2\)CO\(_3\)/2.93 g/l NaHCO\(_3\) (pH 9.6)] for 16 h at 4 °C. After two washes with washing buffer [10 mM PBS (pH 7.4)/0.9 g/l NaCl/0.05% (v/v) Tween-20/0.02% (w/v) NaN\(_3\)], Eu\(^{3+}\)-HDL\(_3\) samples (5 mg/ml; 200 \(\mu\)l per well) diluted in 10 mM PBS, pH 7.4, containing 1 g/l EDTA, were added and incubated for 90 min at 25 °C. After six washes, the fluorescence of bound Eu\(^{3+}\) was measured in the presence of enhancement solution (200 \(\mu\)l per well). The fluorescence was measured with a 1234 Delfia\(^{\circledR}\) research fluorimeter (Wallac Oy).

**Competitive solid-phase sandwich fluorescence assay of 24 h-oxidized Eu\(^{3+}\)-LDL on collagen type I and III with HDL\(_1\)**

For the competitive solid-phase fluorescence sandwich assay, polystyrene microritination plates were coated with the proteins (10 \(\mu\)g/ml of type I and III collagen; 200 \(\mu\)l per well) in 10 mM PBS, pH 7.4, at room temperature overnight. After three washes with washing buffer, 100 \(\mu\)l of 10 \(\mug/ml\) 24 h-oxidized Eu\(^{3+}\)-LDL was mixed with 100 \(\mu\)l of 0, 2 and 24 h-oxidized Eu\(^{3+}\)-HDL\(_3\) (0, 0.1, 1, 10, 100 and 1000 \(\mu\)g/ml HDL\(_3\)) and incubated in 10 mM PBS, pH 7.4, for 90 min at room temperature on a shaker. After six washes with washing buffer, Eu\(^{3+}\) was released with enhancement solution (200 \(\mu\)l per well; Wallac Oy) and fluorescence was measured with a Delfia\(^{\circledR}\) research fluorimeter (Wallac Oy).

Statistical evaluation of the data obtained was performed by means of Student’s t-test.

**Electrophoresis and Western blot analysis**

SDS/PAGE [12% (w/v) gel] was performed under non-reducing conditions. Aliquots (15–20 \(\mu\)l) of native or oxidatively modified HDL\(_2\) protein dissolved in sample buffer [0.075 M Tris, pH 8.8, containing 20% (w/v) glycerol and 0.01% (w/v) Orange G (Aldrich)] were applied per lane. Electrophoresis was performed in a Mini Protean II electrophoresis chamber (60 min at 50 mA and 150 V; Bio-Rad). Transfer to nitrocellulose membranes (0.1 \(\mu\)m pore
size; Hoefer Scientific Instruments) was done with an LKB NovaBlot electrophoretic transfer kit (Pharmacia-LKB) for 90 min at 50 mA and 17 V. The transfer solution contained 48 mM Tris, 39 mM glycine, 0.037% SDS and 20% (v/v) methanol. Non-specific binding sites were blocked with TBS [3% (v/v) skimmed milk in 20 mM Tris/90 mM NaCl/1 mM NaNO (pH 7.4)] for 3 h at 25°C. The nitrocellulose membranes were incubated at 4°C overnight with the antibodies against apo-AI, apo-AII, HNE–protein and MDA–protein adducts (1:1000 dilution in TBS). After three washes with TTBS [TBS containing 0.05% (v/v) Tween-20], on the one hand alkaline phosphatase-conjugated anti-(rabbit IgG) (Sigma), which bound to anti-apo-AI, or horseradish peroxidase-labelled anti-(goat IgG) (Dako), which bound to anti-apo-AII, was added for incubation at 25°C for 3 h. After a further washing, bound rabbit IgG was revealed with 0.5 mg/ml 5-bromo-4-chloro-3-indolyl phosphate in 1 M 2-amino-2-methyl-1-propane, pH 10.3, and bound goat antibody was detected with 25 mg of 4-chloro-1-naphthol in 47.5 ml of TBS containing 2.5 ml of ethanol and 100 µl of H2O2. On the other hand, horseradish-peroxidase-labelled anti-(rabbit IgG) (Dako), which bound to anti-HNE–protein and anti-MDA–protein was added for incubation at 25°C for 3 h. After a wash, enhanced chemiluminescence (ECL) of the revealed antibody was detected on a hyperfilm-ECL (Amersham Life Science).

RESULTS

Alterations of HDL and Eu3+-HDL during oxidation

Native HDL and Eu3+-labelled HDL (Eu3+-HDL) were oxidized in the presence of 10 µM CuSO4 at 37°C. After termination of the oxidation at intervals by the addition of EDTA, the degree of modification of oxidized Eu3+-HDL was estimated as its electrophoretic mobility (EM) relative to non-modified or unlabelled native LDL, or by the content of lipid hydroperoxides (Figure 1). Although the EM (relative to native LDL) of Eu3+-HDL modified oxidatively for 1 h was not altered, the content of lipid hydroperoxides increased immediately after initiation of the oxidation, reaching a maximum concentration after 3–4 h. The EM increased slightly after 2 h, followed by a stronger increase up to 4 h, and reached a maximum at 24 h of oxidation, whereas lipid hydroperoxides decreased strongly between 4 and 24 h.

A similar increase in the relative EM and in the content of lipid hydroperoxides during oxidation was obtained with unlabelled HDL under the same conditions of oxidation as for Eu3+-HDL.

Increase in the immune reactivity of HDL with antibodies against apo-AI and apo-AII and against HNE- and MDA-protein adducts during oxidative modification

A Cu3+-mediated oxidation of LDL leads to the degradation of apolipoprotein B. The native epitopes decreased, as verified by a diminished reactivity to antibodies against native apolipoprotein B [16]. To estimate the influence of oxidation of HDL on the immune reactivity of apo-AI and apo-AII, microtitration plates were coated with polyclonal antibodies against apo-AI and apo-AII. The wells were then incubated with Eu3+-HDL samples oxidatively modified to different degrees. The immune reactivity of Eu3+-HDL with antibodies against apo-AI and apo-AII strongly increased (by approx. 80%) within the first 4 h of oxidation. The degree of oxidation of apoHDL was recorded by measuring the EM relative to native LDL (■) or the content of lipid hydroperoxides (●). A total of four independent oxidation experiments with four different preparations of HDL from four different donors was performed. The results are given as percentages, setting either the content of lipid hydroperoxides of 4 h-oxidized Eu3+-HDL3 to 100%. Values are means ± S.D.

Figure 1 Changes in EM and changes in the content of lipid hydroperoxides of HDL during a Cu3+-mediated (10 µM CuSO4) oxidation at different periods up to 24 h.

The degree of modification of apoHDL was recorded by measuring the EM relative to native LDL (■) or the content of lipid hydroperoxides (●). A total of four independent oxidation experiments with four different preparations of HDL from four different donors was performed. The results are given as percentages, setting either the content of lipid hydroperoxides of 4 h-oxidized Eu3+-HDL3 to 100%. Values are means ± S.D.

Figure 2 Immune reactivity of anti-(apo-AI), anti-(apo-AII), anti-(HNE-protein adducts) and anti-(MDA-protein adducts) to oxidized Eu3+-HDL in a solid-phase fluorescence immunoassay.

Eu3+-HDL was oxidized with 10 µM CuSO4 for different periods up to 24 h. After microtitration plates had been coated with antibodies (1 µg per well), oxidized Eu3+-HDL (2 µg per well) samples were applied. A total of four independent oxidation experiments with four different preparations of HDL from four different donors was performed. The results of binding of oxidized Eu3+-HDL to anti-(apo-AI) (○) and anti-(apo-AII) (▲) (upper panel) or anti-(HNE-protein adducts) (◇) and anti-(MDA-protein adducts) (△) (lower panel) are given as percentages, setting the immune reactivity of 24 h-oxidized Eu3+-HDL to 100%. Values are means ± S.D.
Figure 3  Western blot analysis of native and Cu^{2+}-oxidized HDL_{3}

The lipoproteins, oxidized for different periods at 37 °C, were subjected to non-reducing SDS/PAGE [12% (w/v) running gel] and transferred to nitrocellulose membranes. Western blotting was performed with anti-(apo-AI) and anti-(apo-AII) (top panel), anti-(MDA-protein adducts) (middle panel) and anti-(HNE-protein adducts) (bottom panel) (dilutions 1:1000). Horseradish peroxidase-conjugated anti-(rabbit IgG) (dilution 1:1200) for anti-(apo-AI), anti-(MDA-protein adducts) and anti-(HNE-protein adducts) or alkaline phosphatase-conjugated anti-(sheep IgG) (dilution 1:1200) for anti-(apo-AII) were used as the second antibodies.

oxidation, as shown in Figure 2 (upper panel). This increase was expressed more with the antibody against apo-AI than with that against apo-AII; a slight but equal increase with both antibodies was found up to 24 h of oxidation of Eu^{2+}-HDL_{3}.

Simultaneously with the estimation of the immune reactivity against the antibodies against apo-AI and apo-AII the generation of MDA and HNE epitopes on Eu^{2+}-HDL_{3} during oxidation was followed with the respective antibodies (Figure 2, lower panel). After 4 h of oxidation approx. 60% of newly generated aldehydeic epitopes were present on Eu^{2+}-HDL_{3}. The formations of MDA and HNE epitopes during the time course of oxidation were very similar.
Recognition of aggregated and non-aggregated apolipoproteins of oxidized HDL<sub>3</sub>, by antibodies against apo-AI and apo-AII, and against HNE- or MDA-protein adducts in Western blot analysis

Oxidative modification of HDL<sub>3</sub> was shown to cause the aggregation of apo-AI and apo-AII [21]. This experiment was performed to follow the intermolecular and intramolecular cross-linking and aggregation respectively of apo-AI and apo-AII on the one hand, and to study the expression of the aldehydic epitopes on the other. All differently oxidized HDL<sub>3</sub> samples were subjected to electrophoresis on 12% (w/v) denaturing polyacrylamide gels followed by Western blot analysis with the four antibodies used above. As shown in Figure 3 (top panel), anti-(apo-AI) and anti-(apo-AII) recognized the monomers of apo-AI (28 kDa) and the dimers of apo-AII (17 kDa) of native HDL<sub>3</sub>. 0.5–4 h-oxidized HDL<sub>3</sub> with decreasing intensity, but after 24 h of oxidation no monomers of apo-AI and no dimers of apo-AII were seen on the blots. Bands of aggregated apo-AI and apo-AII arose after only 0.5 h of oxidation of HDL<sub>3</sub> in different patterns and with an intensity dependent on the oxidation of HDL<sub>3</sub>. Apo-AII formed tetramers (34 kDa) and hexamers (51 kDa) at the highest levels between 1 and 3 h of oxidation, whereas apo-AI aggregated after 2 h to form one band at the top of the gel, which was most intense with 24 h-oxidized HDL<sub>3</sub>. Bands with a molecular mass of 45 kDa consisting of both apo-AI and apo-AII at a molar ratio of 1:2 were generated between 0.5 and 4 h of oxidation. Bands revealing a molecular mass of 62 kDa represented adducts of apo-AI and apo-AII at a molar ratio of 1:4. They were detected after 2 h of oxidation, expressed at highest levels between 2.5 and 3.5 h and present to a smaller extent at 4 and 24 h of oxidation.

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Table 1  Semi-quantitative evaluation of the distribution of MDA- or HNE-derived epitopes on apo-AI and apo-AII of Cu<sup>2+</sup>-oxidized HDL<sub>3</sub> obtained by Western blot analysis shown in Figure 3

Symbols: ++++, band showing strongest reaction; ++, band showing strong reaction; +, band showing reaction; –, no reaction.

Compartment binding of 24 h-oxidized Eu<sup>3+</sup>-LDL on collagen type I and III with differently oxidized HDL<sub>3</sub>

It has been shown that strongly oxidatively modified LDL bound in vitro to a greater extent to connective-tissue proteins, such as collagen type I, III and V, than did native LDL [16,22]. To study the ability of HDL<sub>3</sub> to prevent the binding of strongly oxidized LDL to collagen, 24 h-oxidized Eu<sup>3+</sup>-LDL (relative EM 3.6) was mixed with differently oxidized HDL<sub>3</sub> in several concentrations and added to microtitation plates coated with type I and type III collagen. After a wash, the fluorescence in the wells was counted.

Table 2 shows the competition of 24 h-oxidized Eu<sup>3+</sup>-LDL with native, 2 h-oxidized and 24 h-oxidized HDL<sub>3</sub> on type I collagen. At each of the three concentrations of competitor applied (10, 100 and 1000 µg), 2 h-oxidized HDL<sub>3</sub> competed best with oxidized Eu<sup>3+</sup>-LDL (10 µg/ml). However, on further oxidation the competing ability of HDL<sub>3</sub> decreased. Furthermore native HDL<sub>3</sub> was also a good competitor.

With type III collagen (Table 2) similar results were obtained to those for collagen type I. Again, 2 h-oxidized HDL<sub>3</sub> was the strongest competitor for the binding of 24 h-oxidized Eu<sup>3+</sup>-LDL.

The results shown were obtained in nine independent experiments with HDL<sub>3</sub> from four preparations from four donors. Although native HDL<sub>3</sub> decreased the binding of 24 h-oxidized Eu<sup>3+</sup>-LDL to the collagen fibres, the 2 h-oxidized HDL<sub>3</sub> was an even better competitor. However, further oxidation of HDL<sub>3</sub> weakened the competitive capability of HDL<sub>3</sub>.
strongly during the first 2 h of oxidation with Cu,
HDL, such as vitamin E, and the carriage of detectable amounts
than that of LDL, owing to the lack of antioxidants in nascent
LDL to collagen phenotypes of the arterial wall.

Cu
of apo-AI 12–80-fold, whereas other divalent cations such as


In the genesis of atherosclerosis a crucial role is attributed to
LDL, HDL and the LDL/HDL ratio as these lipoproteins are
responsible for the transport of cholesterol [23–25]. The present
study has described for the first time the generation of aldehydic
products on apolipoproteins of HDL3 during Cu
-mediated oxidation and investigated the ability of HDL3 and oxidized
HDL3 to prevent the binding of strongly oxidatively modified
LDL to collagen phenotypes of the arterial wall.

It is known that susceptibility of HDL to oxidation is higher
than that of LDL, owing to the lack of antioxidants in nascent
HDL, such as vitamin E, and the carriage of detectable amounts
of lipid hydroperoxides [26]. Lipid hydroperoxides increased
strongly during the first 2 h of oxidation with Cu
, whereas only
a small change in the relative EM of HDL3 was obtained.

The immune reactivity of HDL3 with the antibodies against
apo-AI and apo-AII strongly increased within the first 4 h of
oxidation. This enhanced immune reactivity might be due to the
presentation of epitopes on the surface of HDL3 that might have
been buried in the lipid domain of this lipoprotein before
oxidation. An increased immune reactivity of apo-AI with storage
of human serum has been described [27]; atmospheric oxidation
was assumed to be the cause. The oxidation of HDL with
Fe
/Fe
or Mn
was found to increase the immune reactivity of
apo-AI 12–80-fold, whereas other divalent cations such as
Cu
at 1 mM had minimal effects [28]. These results are in
contrast with our results, yet it has to be taken into consideration
that we used a concentration of 10 µM Cu
 to achieve the effects
described. Furthermore we also found an increase in the immune
reactivity with anti-(apo-AII), which preceded the increase with
anti-(apo-AI).

In addition to antibodies against apo-AI and apo-AII, we used
antibodies against HNE- and MDA-modified apolipoproteins
for the investigation of oxidatively modified HDL3. HNE and
MDA are breakdown products of lipid hydroperoxides and are
highly reactive, especially with positively charged amino acids
such as lysine, forming aldehydic epitopes [29]. The antibodies
used in this study were made against HNE- or MDA-modified
LDL [30]. However, they also reacted with HDL and other
proteins such as albumin after their modification with the
respective aldehydes [19]. These results suggest that the antibodies
are recognizing epitopes in general formed as HNE- or MDA-
protein adducts. Both antibodies showed a strong increase in the
immune reactivity with oxidatively modified HDL3 within the
first 4 h of oxidation, which was accompanied by an increase in
EM relative to native HDL3. It should be mentioned that Cu
-oxidation or modification of HDL by aldehydes such as HNE,
MDA or others were found to lead to an inhibition of the
activation of lectin:cholesterol acyltransferase [14,15], but the
formation of HNE- or MDA-derived epitopes on oxidation of
HDL3, as obtained in the present study on apo-AI and apo-AII
and their aggregates, was not shown. After only 2 h of oxidation
of HDL3 with Cu
, more than 30 %, of the totally formed new
epitopes were present. To characterize the distribution of the
aldehydic epitopes on the apolipoproteins, Western blot analysis
was performed. HNE-derived epitopes were present on all apo-
AI or apo-AII monomers or polymers except apo-AII dimers,
whereas MDA-derived epitopes could be detected only on apo-
AII tetramers and hexamers, apo-AI polymers and very weakly
on mixed apo-AI/AII (1:2) aggregates. The reason that MDA-
derived epitopes were present on neither apo-AI monomers nor
apo-AII dimers might be that MDA, as a bivalent aldehyde,
leads to cross-linking of the apolipoproteins, whereas HNE is
a monovalent aldehyde.

Studies on the binding of HDL or oxidized HDL to extracellular
matrix were of special importance because in an investigation
by Vollmer et al. [30] a few deposits of apo-AI and apo-
AII in the intima were observed with the first signs of atherosclerosis.
Apo-AI and apo-AII were localized extracellularly, associated with
the connective tissue. With progression of atherosclerosis the distributions of apo-AI, apo-AII and of apolipoprotein B changed. Apo-AI and apo-AII were located within the intimal layer intracellularly, mainly in foam cells, or extracellularly, whereas apolipoprotein B was found to be exclusively deposited extracellularly. Thus it was of interest to investigate in vitro whether HDL3 or oxidized HDL3 was able to bind to matrix proteins, especially to different collagen phenotypes such as LDL and oxLDL [16]. It has been assumed [31] that binding of negatively charged macromolecules such as LDL to the extracellular matrix could be followed by oxidation of the lipoprotein. Afterwards it would be taken up by macrophages via the scavenger receptor’s gliding along the collagen fibrils. Although an increased binding of oxidized LDL to collagen type I, III and V, which tended to increase with the progression of atherosclerosis, has been reported [16,22], little is known about native and oxidatively modified HDL preventing the binding of oxidized LDL to those collagen phenotypes. At the moment we do not have an explanation why 2 h-oxidized HDL3 competed with oxidized LDL for collagen type I and III better than native or 24 h-oxidized HDL3 did, nor why this effect was stronger with collagen type III than with type I. The observations underline the assumption that certain newly formed epitopes on moderately oxidized HDL might be responsible for competition rather than a sole increase in negative charge of oxidized HDL.

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REFERENCES

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Oxidation of high-density lipoprotein and binding to matrix proteins


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