Human high density lipoproteins (HDL) can reduce cholesteryl ester hydroperoxides to the corresponding hydroxy derivatives (Sattler W., Christison J. K., and Stocker, R. (1995) Free Radical Biol. & Med. 18, 421–429). Here we demonstrate that this reducing activity extended to hydroperoxides of phosphatidylcholine, was similar in HDL2 and HDL3, was independent of arylesterase and lecithin:cholesterol acyltransferase activity, was unaffected by sulfhydryl reagents, and was expressed by reconstituted particles containing apoAI or apoAII only, as well as isolated human apoAI. Concomitant with the reduction of lipid hydroperoxides specific oxidized forms of apoAI and apoAII formed in blood-derived and reconstituted HDL. Similarly, specific oxidized forms of apoAI accumulated upon treatment of isolated apoAI with authentic cholesteryl linoleate hydroperoxide. These specific oxidized forms of apoAI and apoAII have been shown previously to contain Met sulfoxide (Met(O)) at Met residues and are also formed when HDL is exposed to Cu2+ or soybean lipoxygenase. Lipid hydroperoxide reduction and the associated formation of specific oxidized forms of apoAI and apoAII were inhibited by solubilizing HDL with SDS or by pretreatment of HDL with chloramine T. The inhibitory effect of chloramine T was dose-dependent and accompanied by the conversion of specific Met residues of apoAI and apoAII into Met(O). Canine HDL, which contains apoAI as the predominant apolipoprotein and which lacks the oxidant-sensitive Met residues Met112 and Met148, showed much weaker lipid hydroperoxide reducing activity and lower extents of formation of oxidized forms of apoAI than human HDL. We conclude that the oxidation of specific Met residues of apoAI and apoAII to Met(O) plays a significant role in the 2-electron reduction of hydroperoxides of cholesteryl esters and phosphatidylcholine associated with human HDL.

Plasma levels of high density lipoprotein (HDL) cholesterol and apolipoprotein (apo)A-I inversely correlate with the risk of developing coronary heart disease (1, 2). This is generally thought to be due to the participation of HDL in removal of cholesterol from peripheral tissues and its ensuing transport to the liver for excretion (3). HDL has other potential anti-atherogenic properties, such as its ability to inhibit the oxidation of low density lipoproteins (LDL) (4) and the pro-atherogenic activities of oxidized LDL (5, 6). LDL oxidation appears to be a key event in atherogenesis (see e.g. Ref. 7), and oxidized lipids, particularly those of cholesteryl esters, are present in human atherosclerotic lesions (8).

HDL, but less so LDL, can reduce cholesteryl ester hydroperoxides (CE-OOH) to the corresponding cholesteryl ester hydroxides (CE-OH) (9). We have proposed (9, 10) that this reducing activity of HDL is potentially anti-atherogenic based on the following: (i) by reducing CE-OOH to CE-OH, HDL converts potentially reactive species to relatively inert species, less likely to give rise to further radicals and hence secondary radical reactions (9); (ii) HDL also reduces CE-OOH transferred from oxidized LDL via cholesteryl ester transfer protein (11) and thus can potentially detoxify pro-atherogenic oxidized LDL; (iii) the reduction of HDL CE-OH occurs in and is selectively accelerated by rat liver perfusate (10); (iv) HDL CE-OH is rapidly and selectively removed by Hep G2 cells in culture (12) and in situ perfused rat liver (10); and (v) in rats, HDL CE-OH are rapidly cleared from the circulation by the liver and secreted into bile (13), indicating a physiologically plausible (3) detoxification and exit route for oxidized lipids associated with HDL.

The underlying mechanism(s) for HDL's CE-OH reducing activity has not been elucidated. Here we report evidence for lipid hydroperoxides (LOOH) being reduced by specific Met residues of apoAI and apoAII in HDL.

EXPERIMENTAL PROCEDURES

Materials—Chloramine T, phenylacetate, 5,5'-dithiobis-2-nitrobenzoinic acid (DTNB), and p-chloromercuriphenylsulfonic acid (PCMS) were purchased from Sigma. All other chemicals, solvents, and materials were obtained from the sources indicated in the accompanying paper (14).

Isolation of HDL and ApoAI—Human and canine HDL and human HDL2 and HDL3 were isolated rapidly from freshly obtained EDTA plasma by the 4-h two-step density gradient ultracentrifugation using a TL100.4 rotor (Beckman Instruments, Palo Alto, CA) described previ-
Reducing activity and arylesterase activity were assessed in parallel. Forms were determined by HPLC as described (14). Detection was used. Unoxidized apoAI and apoAII and their oxidized oxonase/arylesterase (EC 3.1.8.1) activity was studied, HDL was isolated from hemopurinized human plasma into 50 mM Tris/HCl, pH 7.4, and not exposed to either phosphate buffer or EDTA unless specifically indicated. HDL protein concentrations were estimated and apoI isolated as described (15).

Preparation of Spherical Reconstituted HDL—Spherical reconstituted HDL containing apoAI only (3 molecules of apoAI/particle, rHDL-AI) or apoAII only (6 molecules of apoAII dimer/particle, rHDL-AI) were generated by incubating discoidal rHDL prepared by cholate dialysis, with purified lecithin:cholesterol acyltransferase (LCAT) and LDL (16). As judged by non-denaturing polyacrylamide gradient gel electrophoresis, the rHDL-AI and rHDL-AII each comprised a single, homogeneous population of particles. The molar ratio of phospholipids/cholesterol esters/unesterified cholesterol/protein of the particles were 32.4:18.9:5.0:1 for rHDL-AI and 32.6:19.1:5.1:2 for rHDL-AII. All compositional analyses were performed on a Cobas Fara autoanalyzer (Roche Diagnostics, Switzerland). The stoichometry varied by <5% for all components in two independent preparations of each rHDL. The majority of the cholesteryl esters was cholesterol palmitate and cholesteryl oleate; however, cholesteryl linoleate was also present at ~0.65 mol/mol unesterified cholesterol, presumably derived from LDL during the conversion of the discoidal rHDL to spherical rHDL-AI (16).

HDL Reducing Activity—Isolated HDL (1.5–2.0 mg of protein/ml) was oxidized in PBS containing 1 mM EDTA by aerobic incubation for 2 h at 37 °C in the presence of AAPH (4–7.5 mM), a generator of aqueous peroxyl radicals. After oxidation, AAPH was removed by gel filtration and HDL supplemented with 1 mM EDTA, unless specified otherwise. To selectively oxidize Met residues (17), HDL (~1.5 mg of protein/ml) was incubated for 1 h at 22 °C in PBS, 1 mM EDTA and in the presence of chloramine T (50–500 μM). Unreacted chloramine T was removed subsequently by gel filtration and HDL was then either analyzed immediately or oxidized with AAPH as above, the radical generator removed and CE-OOH reducing activity assessed by incubation at 37 °C in the presence of 1 mM EDTA under argon and in the dark. In other experiments HDL was pretreated with H2O2 (1–200 μM) or HOCl (50–500 μM) instead of chloramine T. HDL (~1.5 mg of protein/ml) was also incubated for 4 h at 22 °C in PBS (1 mM EDTA) with 2 mM DTNB or 2 mM PCMP to derivatize thiol associated with the lipoprotein. Since apoAI does not contain Cys and the sole Cys residue of apoAII forms a disulfide giving rise to the apoAII homodimer, the major targets for these reagents were most likely to be the quantitatively minor HDL apolipoproteins and HDL-associated proteins, notably LCAT.

Loss of endogenous fluorescence of Tryp residues was determined in SDS-solubilized HDL or different apoAII forms after HPLC separation (14). Analysis of HDL's unoxidized lipids, CE-OOH, CE-OH, and phosphatidylcholine hydroperoxides (PC-OOH), was by HPLC as described (14). For determination of PC-OOH plus phosphatidylcholine hydroxides (PC-OH) HPLC, with UV 234 nm instead of chemiluminescence detection was used. Unoxidized apoAI and apoAII and their oxidized forms were determined by HPLC as described (14).

Measurement of HDL Arylesterase Activity—HDL (20–40 μg of protein), isolated in the absence of EDTA, was added to 950 μl of 25 mM Tris/HCl, 1 mM CaCl2, 1 mM phenylacetate, and the increase in absorbance at 270 nm was recorded for at least 60 s (6, 18). Arylesterase activity was calculated using the molar extinction coefficient of 1,310 M−1 cm−1 and defined as 1 μmol of phenylacetate hydrolyzed per min, with the specific activity given as units/mg HDL protein. Arylesterase activity was inhibited by pretreating HDL with 3 mM EDTA for 90 min at 37 °C under argon (6). EDTA was then removed by gel filtration, the HDL oxidized, and CE-OOH and PC-OOH reduction assessed as described above but in 25 mM Tris/HCl, pH 8.0, containing 1 mM CaCl2. Reducing activity and arylesterase activity were assessed in parallel with HDL or “chelated HDL” in the presence of 1 mM CaCl2 or 1 mM EDTA, respectively. Esterase activity was determined before and after AAPH-induced oxidation of HDL and at the end of the time course experiments where CE-OOH and PC-OOH reduction was also assessed.

Fast Protein Liquid Chromatography—HDL (1.2 mg of protein in 200 μl), in the absence and presence of 1% SDS, was subjected to size exclusion chromatography using a fast protein liquid chromatography system (Pharmacia Biotech Inc.) fitted with a Superose-12 (Pharmacia) column (30 × 1.5 cm inner diameter) eluted with 20 mM sodium phosphate buffer (pH 7.8; 4 °C) at 0.25 ml/min and 279 nm.

RESULTS

Reduction of HDL CE-OOH and PC-OOH—By using radio-labeled CE-OOH, we have demonstrated previously that HDL (much more effectively than LDL) reduces this class of LOOH in a stoichiometric fashion to CE-OH (9). We sought here to confirm this and in addition to determine whether PC-OOH was also reduced by HDL incubated at 37 °C. For this, we first mildly oxidized HDL using AAPH and assessed reduction of CE-OOH and PC-OOH subsequent to removal of AAPH (see “Experimental Procedures”). In agreement with previous results (9), the proportion of total oxidized cholesteryl esters present as CE-OH increased as time progressed (Fig. 1A), and there was a near stoichiometric conversion of CE-OOH to CE-OH (Fig. 1A, inset). Fig. 1B shows, for the first time, that the proportion of total oxidized phosphatidylcholine present as phosphatidylcholine hydroxides (PC-OH) also increased as
time progressed. By contrast, the levels of PC-OOH decreased rapidly and those of PC-OH plus PC-OOH (i.e. PC-O(O)H) remained unaltered (Fig. 1B, inset), indicating that PC-OOH was reduced to PC-OH.

The addition of 1% (w/v) SDS to HDL inhibited both CE-OOH and PC-OOH reduction (Fig. 1). The presence of SDS disrupted the structural integrity of HDL. Thus, in the absence of SDS, a single major peak, corresponding to native HDL particles, eluted from the gel filtration column, whereas in the presence of 1% SDS, a series of broad and ill-defined peaks were observed (Fig. 2). This suggests that an intact HDL particle was required in order for both CE-OOH and PC-OOH reduction to occur efficiently. Studies performed using HDL$_A$ and HDL$_B$ subfractions showed that the CE-OOH reducing activity of both subfractions was similar to that of HDL (Fig. 3).

To understand the mechanism(s) by which HDL reduces CE-OOH and PC-OOH, we first investigated a possible contribution of HDL-associated paraoxonase/arylesterase (EC 3.1.8.1). This enzyme hydrolyzes aryl esters of carboxylic acids and organophosphates (18) and oxidized (but not native) fatty acyl chains of phospholipids (6), requires Ca$^{2+}$ for its activity, and is inhibited effectively by EDTA (19). We therefore treated isolated HDL with EDTA (referred to as chelated HDL) and determined its impact on CE-OOH and PC-OOH reducing and arylesterase activities. Chelated HDL was almost totally devoid of arylesterase activity (Fig. 4A, yet maintained its CE-OOH or PC-OOH reducing activity (Fig. 4, B and C). Arylesterase activity in freshly isolated HDL was 5.2 ± 1.7 units/mg (mean ± S.D., n = 3) and decreased to 45 ± 12% of that (mean ± SD, n = 3) after AAPH-induced oxidation and a further 19 h incubation at 37 °C, demonstrating that although the absolute activity was lower, arylesterase remained active throughout the entire time course studied.

In separate experiments, HDL was incubated with 2 mM DTNB to inhibit the activity of HDL-associated LCAT. DTNB oxidizes Cys residues which are thought to be critical for enzyme activity (20), and others have shown that 0.2–1.5 mM DTNB inhibits LCAT activity (see e.g. Ref. 21). However, treatment of HDL with 2 mM DTNB had no discernible effect on subsequent CE-OOH reducing activity (Fig. 5). Similarly, PC-MPS, another LCAT inhibitor, also failed to affect CE-OOH reducing activity (Fig. 5). Thus, neither arylesterase nor LCAT activities play a role in the CE-OOH or PC-OOH reducing activity of HDL.

To prove unambiguously that HDL’s apolipoproteins rather than associated enzymes/proteins support the observed reducing activity, we tested CE-OOH reduction by rHDL$_{AI}$ and rHDL$_{AII}$. The purity of the protein composition of these rHDL was confirmed by SDS-polyacrylamide gel electrophoresis with silver staining (data not shown) and by reversed phase HPLC. rHDL$_{AI}$ contained only apoAI, whereas rHDL$_{AII}$ contained apoAI with traces (<5%) of apoAI, and both particles clearly reduced CE-OOH to CE-OH, with rHDL$_{AII}$ being somewhat more efficient than rHDL$_{AI}$ (Fig. 6). These results imply that apoAI and apoAII are responsible for the LOOH reducing activity associated with native human HDL. Since apoAI does not contain Cys and apoAII does not contain Trp, these results also demonstrate that Cys and Trp are not essential for CE-OOH reduction. Cys is thought to be present almost exclusively as a disulfide in apoAII, giving rise to the homodimeric structure of human apoAII (22).

Oxidation of Met Residues of ApoAI and ApoAII Is Associated with the Reduction of LOOH by HDL—Previous reports have documented the sensitivity of certain Met residues of apoAI and apoAII to oxidation (14, 17, 23). Based on this evidence, we speculated that Met oxidation in apoAI and apoAII might be responsible for the reduction of LOOH. To investigate this possibility, pre-oxidized HDL was incubated at 37 °C under argon (i.e. conditions that support CE-OOH and PC-OOH reducing activity) and the formation of oxidized forms of both apoAI and apoAII followed by HPLC (see “Experimental Procedures”). A representative chromatogram of apolipoproteins in native HDL is given in Fig. 7A. As shown previously (14, 17, 23), apoAI and apoAII were the major apolipoproteins in HDL, with apoCs as minor components.

The accompanying paper (14) describes the time-dependent decrease in the content of apoAI and apoAII and concomitant formation apoAI$_{16}$, apoAI$_{32}$, and apoAII$_{16}$ during AAPH-induced oxidation of HDL. ApoAII contains a single Met(O) in place of one of the two Met$^{26}$ residues of apoAI dimer (23), whereas apoAI$_{16}$ and apoAII$_{32}$ contain one and two Met(O) residues per apoAI monomer, respectively (14, 17). The chromatogram shown in Fig. 7B is derived from pre-oxidized HDL and is representative of changes occurring to apoAI and apoAII when the intact lipoprotein is exposed AAPH for restricted periods (14). The observed specific formation of apoAI$_{16}$, apoAI$_{32}$, and apoAII$_{16}$ during exposure of HDL to AAPH is not due to direct oxidation of the apolipoproteins by AAPH-derivatives (14). Incubation of such pre-oxidized HDL subsequent to the removal of all AAPH resulted in a further time-dependent decrease in unoxidized apoAI and apoAII and a concomitant increase in the formation of apoAI$_{16}$, apoAI$_{32}$, and apoAII$_{16}$ (Fig. 7, B–D). Inclusion of 1% (w/v) SDS significantly inhibited the formation of oxidized forms of apoAI and apoAII (Fig. 8), to an extent comparable with the inhibition of LOOH reduction (cf. Fig. 1).

We next confirmed that formation of the oxidized forms of apoAI and apoAII occurred concomitantly with HDL LOOH reduction in both rHDL$_{AI}$ and rHDL$_{AII}$. As shown in Fig. 9, the
Inhibition of CE-OOH Reduction by Pre-oxidation of Met Residues of ApoAI and ApoAII—In the absence of sulfhydryl groups, chloramine T selectively oxidizes Met residues (24), including Met$^{112}$ and Met$^{148}$ in apoAI of intact HDL (17). To investigate the role of apoAI and apoAII Met residues in CE-OOH reduction, we pretreated HDL with chloramine T before pre-oxidation with AAPH and subsequent assessment of CE-OOH reducing activity. As expected, treatment of HDL with increasing concentrations of chloramine T caused an increased proportion of the Met(O)-containing oxidized forms of apoAI and apoAII (Fig. 1A), reminiscent of the situation with AAPH-

oxidized HDL incubated at 37 °C after removal of the radical generator. There was no discernible decrease in the Trp fluorescence in the chloramine-T-oxidized samples, as indicated by unaltered ratios of UV$^214$ nm absorbance to Trp fluorescence in the unoxidized and oxidized forms of apoAI after HPLC separation (see “Experimental Procedures”). In addition, Trp fluorescence in intact HDL directly after treatment with 50, 100, and 500 μM chloramine T was 92 ± 9, 94 ± 4, and 90 ± 5% (mean ± S.D., n = 4) of the nontreated, native HDL, respectively. This indicated that Trp residues in apoAI were not appreciably oxidized upon treatment of HDL with chloramine T. Furthermore, such treatment did not result in severe lipid damage. Thus, even at the highest chloramine-T concentration used, the levels of cholesteryl linoleate and α-tocopherol were ~95 and ~75% of control values. Importantly, there was a dose-dependent decrease in both the levels of CE-OH formed during the 2-h pre-oxidation period and the CE-OOH reducing activity when HDL was treated with increasing concentrations of chloramine T (Fig. 1B). This effect was already evident at the zero time point as illustrated by the lower degree of CE-OH detected at the higher chloramine-T concentrations used (Fig. 1B).

FIG. 5. Treatment of HDL with LCAT activity inhibitors does not inhibit CE-OOH reducing activity. HDL (~1.5 mg of protein/ml) was incubated for 4 h at 22 °C in PBS containing 1 mM EDTA and, where indicated, 2 mM PCMPS or 2 mM DTNB. The thiol-reactive reagents were then removed and HDL pre-oxidized with 7.5 mM AAPH for 2 h at 37 °C, AAPH removed, and CE-OOH reducing activity assessed as described under “Experimental Procedures.” Data represent control HDL not treated with LCAT inhibitor (●) or HDL treated with DTNB (○) or PCMPS (□).

FIG. 6. Reduction of CE-OOH by rHDL containing either apoAI or apoAII. rHDL containing only apoAI (rHDL$_{AII}$) or apoAII (rHDL$_{AII}$) were prepared, pre-oxidized with 7.5–10 mM AAPH for 2 h at 37 °C, and subsequently assessed for CE-OOH reducing activity, as described under “Experimental Procedures.” The initial CE-OOH concentrations for both rHDL were ~5 μM. Data are means ± range of two experiments using separate preparations of rHDL.
HDL’s apoAI and apoAII results in a dose-dependent inhibition of subsequent LOOH reducing activity.

**CE-OOH Reducing Activity of Canine HDL**—If Met residues of apoAI and apoAII were important for the CE-OOH reducing activity associated with HDL, the prediction would be that isoforms of apoAI and apoAII lacking Met112 and Met148 would exhibit decreased CE-OOH reducing activity. ApoAI is the predominant apolipoprotein in canine HDL (Ref. 25, verified by SDS-polyacrylamide gel electrophoresis, data not shown) and shares \(85\%\) sequence homology with human apoAI (29), and Met112 and Met148 are substituted by Val and Leu, respectively (29). Oxidation of canine HDL (2.0 mg of protein/ml) with AAPH (7.5 mM for 2 h at 37 °C) resulted in similar initial levels of CE-OOH in the reaction mixtures compared with human HDL (10.2 \(\pm\) 1.3 \(\mu \)M, mean \(\pm\) range, \(n = 2\) versus 13.1 \(\pm\) 2.1, mean \(\pm\) S.D., \(n = 6\), for canine versus human HDL, respectively). In contrast, CE-OOH reducing activity was substantially decreased, although not absent (Fig. 11A). The low degree of CE-OOH reduction observed in canine HDL was comparable with that seen in human HDL in the presence of 1% (w/v) SDS (cf. Fig. 1); it was accompanied by a depletion of native canine apoAI and the formation of a less hydrophobic form of apoAI (data not shown). Although we have not characterized this new (oxidized) form, canine apoAI was clearly less reactive than apoAI of human HDL (Fig. 11B).

**CE-OOH Reducing Activity of Isolated ApoAI**—To test whether formation of Met(O) in HDL is due to reaction of Met112 and Met148 with CE-OOH directly, thus yielding CE-OH, we incubated isolated apoAI with CE-OOH. This resulted in a time-dependent decrease in the concentration of CE-OOH while CE-OH accumulated (Fig. 12A). A linear approximation of the decay curve (Fig. 12B) showed that CE-OOH reduction is proportional to the initial concentration of Met, indicating that Met112 and Met148 are the primary sites of CE-OOH attack in apoAI.
showed that the rate of CE-OH formation was ~64% of the rate of CE-OOH depletion. The absence of a strict 1:1 stoichiometry may be explained by the formation of products other than hydroxides. Exposure of isolated apoAI to CE-OOH also resulted in an increase in apoAI-C (26) and apoAI-1, the latter as a shoulder prior to the unoxidized apoAI peak (not shown). Under these conditions, the rate of apoAI-CE-OH formation was not correlated quantitatively to the rates of CE-OOH depletion and CE-OH accumulation. Irrespective of the issue of quantitation, however, these results demonstrate unequivocally that Met112 and Met148 in apoAI can reduce CE-OOH to CE-OH. It was found necessary to have CE-OOH present in excess to apoAI to obtain an appreciable rate of product formation. This was in contrast to the experiments with native HDL where the molar ratio of apoAI/CE-OOH was ~3:1 (e.g., for Figs. 3 and 7). It is conceivable that the native structure of apoAI in HDL facilitates a direct interaction with the peroxide moiety of CE-OOH due to immediate apposition of the two reactive groups, consistent with the much lower rate of CE-OOH reduction in SDS-treated versus intact HDL (see Fig. 1). In any case, CE-OOH were not appreciably reduced to CE-OH during the course studied when apoAI was replaced with the same mass of bovine serum albumin or when the peroxide was incubated in the absence of apoAI (not shown).

DISCUSSION

The present study strongly supports a role for Met residues of apoAI and apoAII as the principal mechanism by which HDL reduces LOOH to lipid hydroxides. Under all circumstances examined, the reduction of LOOH by HDL was accompanied by the formation of specifically oxidized apoAI and apoAII that contained Met(O) as the selective oxidation product. Also, selective oxidation of HDL’s Met residues with nucleophilic oxidants such as chloramine T inhibited subsequent LOOH reducing activity in a dose-dependent manner. Furthermore, canine HDL, the apoAI of which lacks the oxidation-sensitive Met112 and Met148 (26), showed much less reducing activity. Finally, apoAI in solution was capable of reducing CE-OOH to CE-OH with concomitant formation of Met(O). Together, these results provide evidence for Met112 and Met148 of apoAI and Met26 of apoAII oxidizing to Met(O) as LOOH are reduced to the corresponding lipid hydroxides in HDL (Scheme 1).

The possibility that HDL-associated proteins other than apolipoproteins are responsible for the observed reduction of LOOH was addressed using several different approaches. Arylesterase activity was inhibited by EDTA treatment, yet this had no inhibitory effect on LOOH reduction (Fig. 4). Also, the lack of effect of treatment of HDL with DTNB or PCMPS (Fig. 5) indicated that neither LCAT (27) nor accessible thiols are required for the LOOH reducing activity. A potentially relevant activity associated with HDL not investigated here is the trypanosome lytic factor, which has been reported to exhibit a peroxidase-like activity in the presence of H2O2 (28). However, this activity is located almost exclusively on the very dense subfractions of human HDL and is largely absent from HDL2, whereas HDL2 and HDL3 showed equal CE-OOH reducing activity (Fig. 3). Therefore, a role for trypanosome lytic factor in LOOH reduction by HDL is also not likely. Finally, our observation that rHDL containing either apoAI or apoAII alone fully supports LOOH reduction demonstrates unambiguously that both of the major apolipoproteins of human HDL are able to reduce LOOH, independent of any other protein activity.
inhibited LOOH-induced Met oxidation, that the CE-OOH reducing activity of HDL is much greater than that of LDL (9), that canine HDL has low reducing activity, and that only Met\textsuperscript{112} and Met\textsuperscript{148} but not Met\textsuperscript{86} residue(s) in apoAI become oxidized during LOOH reduction (14, 17, 23).

The finding that canine HDL, which lacks the oxidation-sensitive Met\textsuperscript{112} and Met\textsuperscript{148} of human apoAI, exhibited only slight CE-OOH reducing activity (Fig. 11) is consistent with our hypothesis that these Met residues are of significant importance. The remaining low reducing activity of canine HDL may be due to an interaction of LOOH with other redox-active amino acids, possibly including Met\textsuperscript{86}, although previous studies have shown that it is resistant to autoxidation (17) and AAPH-induced oxidation of human HDL.\textsuperscript{2}

The fact that Met(O) formed in proteins can be reduced by peptide Met(O) reductase (EC 1.8.4.6) (24, 35, 39) raises the possibility that apoAI and apoAII could act as redox-cycling “active sites” in a pseudo-enzymatic way, providing a peroxidase-like activity. The implied reduction of HDL’s LOOH to lipid hydroperoxides by the proposed 2-electron reaction (Scheme I) would represent an antioxidant defense, as it prevents the participation of LOOH in secondary radical or other potentially damaging reactions. Peptide Met(O) reductase, present in virtually all mammalian tissues (37), is assumed to be an intracellular enzyme, consistent with its requirement for thiol reduction and thiol reductase (38), and the fact that it reduces Met(O) in extracellular α-1-proteinase inhibitor in vitro (38) but not in blood (39). Future studies will show whether Met(O) reductase is capable of reducing Met(O) formed in apoAI and apoAII and thereby regenerate HDL’s LOOH reducing activity.

On the other hand, if extracellular oxidation of Met to Met(O) in HDL is not a reversible process in biological systems, its formation can be considered as oxidative damage, and apoAI/AII containing Met(O) would be expected to accumulate at sites of oxidative stress. Consistent with this, lipoprotein fractions of HDL, density-isolated from advanced human atherosclerotic plaques by ultracentrifugation, contain detectable amounts of oxidized isoforms of both apoAI and apoAII.\textsuperscript{2}

The question whether the LOOH reducing activity associated with Met residues of HDL represents an antioxidant defense or oxidative damage has potential ramifications for a number of processes relevant to atherosclerosis. As discussed above, limitation of the progression of oxidative modification of LDL by reductive removal of LOOH is one important example. In addition, in vitro oxidation of HDL by copper can result in a loss of the lipoprotein’s ability to stimulate the efflux of cholesterol from foam cells (40–42), the first step in reverse cholesterol transport. It is possible that the specific protein oxidation resulting from the reduction of LOOH is also sufficient to cause changes in HDL’s ability to promote cholesterol efflux from cells. Of possible significance, Met\textsuperscript{112} and Met\textsuperscript{148} (of apoAI) and Met\textsuperscript{86} (apoAII) are located within the known lipid binding domains of the respective apolipoproteins (43). In addition, the presence of Met(O) in oxidized peptide analogs of apoAI and apoAII alters their secondary structure and affects their ability to interact with lipids (23) which, at least for apoAI, is crucial for both cholesterol removal and activation of LCAT. In light of these considerations and our present findings, it seems possible that selective oxidation of Met residues, as can be expected whenever lipid peroxidation occurs, may affect the biological activities of HDL.

In summary, the present studies provide evidence that the ability of HDL to reduce and thereby detoxify potentially pro-
atherogenic LOOH is due, to a significant degree, to a reaction with specific Met residues on apoAI and apoAII.

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