Apolipoprotein A-I Promotes the Formation of Phosphatidylcholine Core Aldehydes That Are Hydrolyzed by Paraoxonase (PON-1) during High Density Lipoprotein Oxidation with a Peroxynitrite Donor*

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High density lipoprotein (HDL) is rich in polyunsaturated phospholipids that are sensitive to oxidation. However, the effect of apolipoprotein A-I and paraoxonase-1 (PON-1) on phosphatidylcholine oxidation products has not been identified. We subjected native HDL, trypsinized HDL, and HDL lipid suspensions to oxidation by the peroxynitrite donor, 3-morpholinosydnonimine. HDL had a basal level of phosphatidylcholine monono- and di-hydroperoxides that increased to a greater extent in HDL, compared with either trypsinized HDL or HDL lipid alone. Phosphatidylcholine core aldehydes, which were present in small amounts, increased 10-fold during oxidation of native HDL, compared with trypsinized HDL (p = 0.004), and 4-fold compared with HDL lipid suspensions (p = 0.0021). In addition, the content of lysophosphatidylcholine increased 300% during oxidation of native HDL, but only 80 and 25%, respectively, during oxidation of trypsinized HDL and HDL lipid suspensions. Phosphatidylcholine isoprostanes accumulated in comparable amounts during the oxidation of all three preparations. Incubation of apolipoprotein A-I with 1-palmitoyl-2-linoleoyl glycerophosphocholine proteoliposomes in the presence of 3-morpholinosynonimine or apoAI with phosphatidylcholine hydroperoxides resulted in a significant increase in phosphatidylcholine core aldehydes with no formation of lysophosphatidylcholine. We propose that apolipoprotein A-I catalyzes a one-electron oxidation of alkoxyl radicals. Purified PON-1 hydrolyzed phosphatidylcholine core aldehydes to lysophosphatidylcholine. We conclude that, upon HDL oxidation with peroxynitrite, apolipoprotein A-I increases the formation of phosphatidylcholine core aldehydes that are subsequently hydrolyzed by PON1.

There is significant evidence that the role of lipoproteins in cardiovascular disease involves oxidation of the lipid-protein complex (1). The oxidative susceptibility and products of the oxidation of low density lipoprotein (LDL)1 have been the most intensively studied area to date (2, 3). However, it is known that the other major lipoprotein complexes, the very low density lipoproteins (4, 5) and the high density lipoproteins (HDL) (6–8), can also undergo oxidation. Within nature, protection of polyunsaturated fatty acids from oxidation has come in the form of water-soluble antioxidant molecules, such as vitamin C, and lipid-soluble anti-oxidant molecules, such as vitamin E. Goulinet and Chapman (9) have shown that there is a marked gradient of the tocopherol and carotenoid classes of lipid-soluble antioxidants among the major lipoproteins. Very low density lipoproteins, LDL, and HDL were shown to have an average of 43, 10, and 0.7 antioxidant molecules per particle, respectively. On this basis alone, one would predict that HDL would be the lipoprotein complex most susceptible to oxidation. Indeed, HDL is as susceptible or more susceptible to oxidation in vitro than is LDL (7). However, HDL has been shown to protect LDL from oxidation in vitro (10). The factors protecting against formation of lipid hydroperoxides in plasma appear to be apoA-I (11–13) and the enzyme paraoxonase (PON-1) (10, 14).

Peroxynitrite, the product of nitric oxide and superoxide, is thought to be an important biologically produced oxidant (15, 16). It is relevant to cardiovascular disease, as its formation is enhanced by inflammatory responses of macrophages and neutrophils, and in conditions such as ischemia reperfusion (16, 17). During an inflammatory response, acute phase HDL is formed, which itself becomes proinflammatory, in contrast to the anti-inflammatory properties of native HDL (18, 19). This acute phase HDL may be oxidatively modified by peroxynitrite. Peroxynitrite can directly oxidize polyunsaturated fatty acids (16), tocopherols (20–22), carotenoids (20), proteins, carbohydrates, and DNA (16). As reviewed by Francis (7), a number of

1 The abbreviations used are: LDL, low density lipoproteins; apo, apolipoprotein; DMPC, dimyristoyl glycerophosphocholine; DTDP, N-bis(carboxymethyl)amino-ethylglycinepentaaacetic acid; HFEA, heptfluorobutyric acid; HDL, high density lipoproteins; LC/ESI/MS, liquid chromatography/electrospray ionization/mass spectrometry; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; BHT, butylated hydroxytoluene; HPLC, high pressure liquid chromatography; C5 Acid PC, 1-palmitoyl-2-glu-taryl-sn-glycero-3-phosphocholine; C9 Acid PC, 1-palmitoyl-2-(9-oxo)nonanoyl-sn-glycero-3-phosphocholine; C9 Ald PC, 1-palmitoyl-2-(9-oxo)nonanoyl-sn-glycero-3-phosphocholine; C9 Acid PC, 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine; PAPC, 1-palmitoyl-2-arachidonyl glycero-3-phosphocholine.
oxidants have been used to modify HDL. These either primarily modify HDL lipid or HDL proteins and most often impair known functions of HDL. Oxidation of HDL by tyrosyl radical affects primarily HDL apoproteins and enhances its activity in reverse-cholesterol transport (7). However, the oxidation of HDL by peroxynitrite has not been well characterized.

3-Morpholinosydnonimine (SIN-1) generates both nitric oxide and superoxide simultaneously to form peroxynitrite (23). Therefore, it mimics the environment that lipoproteins may be exposed to in the vasculature (15).

We have compared the oxidation products of native HDL, trypsinized HDL, and HDL lipid suspensions and phosphatidylcholine apoA-I proteoliposomes. Our results show that apoA-I increases the formation of phosphatidylcholine core aldehydes. In addition, we observed that lysophosphatidylcholine was formed in significant amounts only during oxidation of intact HDL, consistent with activation of a phospholipase A₂-like activity. We conclude that PON-1 has a phospholipase A₂-like activity toward phosphatidylcholine core aldehydes.

**EXPERIMENTAL PROCEDURES**

**Materials**—SIN-1, N-bis(carboxymethylamino)-ethylglycinepentacetic acid (DTTPA), dipentadecanoylglycerophosphocholine, 1-palmitoyl-2-linoleoyl-sn-glycerol-3-phospholipid (PLPC), 1-palmitoyl-2- arachidonoyl-sn-glycerol-3-phosphocholine (PAPC), and dimyristoyl-sn-glycerol-3-phosphocholine (DMPC) were from Avanti Al (Alabaster, AL); t-butyl-hydroperoxide and trypsin (bovine pancreas) were purchased from Sigma. Polyclonal rabbit anti-human apoA-I and anti-human apoA-II antibodies were prepared in the laboratory (24), and anti-rabbit IgG alkaline phosphatase conjugate was purchased from Bio-Rad. 1-Palmitoyl-2-(5-oxo)valeroyl-sn-glycerol-3-phosphocholine and 1-palmitoyl-2-(9-oxo)nonanoyl-sn-glycerol-3-phosphocholine and the corresponding core acids were prepared in the laboratory by reductive ozonolysis of PLPC and PAPC, as described previously (25). Choline phospholipids were measured using the choline phospholipid enzymatic assay kit from Roche Molecular Biochemicals. All solvents used in liquid chromatography-mass spectrometry were HPLC grade. Other solvents and chemicals were of reagent grade and were provided by local suppliers.

Isolation and Oxidation of HDL—HDL was isolated from serum of subjects fasted for 12–14 h, by ultracentrifugation between densities 1.063 and 1.21 g/ml (26), and exhaustively dialyzed in 10 mM phosphate-buffered saline (PBS), pH 7.4. HDL (1 mg/ml protein) (27) was oxidized by the peroxynitrite donor, SIN-1 (1 mM), for up to 20 h at 37 °C, in the presence of DTTPA (100 μM), a metal ion chelator (28). At each time point, an aliquot was withdrawn; the oxidation was stopped by the addition of 100 μM butylated hydroxytoluene (BHT), and HDL lipid-soluble oxidation products were extracted with chloroform/methanol (1:1) (29). The yields of the oxidation products were expressed as μg/mg of phosphatidylcholine at time 0.

**SDS-PAGE**—SDS-PAGE was performed, in the presence of dithiothreitol, using an 11% polyacrylamide gel (30). After electrophoresis, proteins were either stained, using silver stain (Plus One, Amersham Pharmacia Biotech), or transferred to polyvinylidine fluoride membranes and immunoblotted using polyclonal rabbit anti-human apoA-I or anti-human apoA-II antibodies.

**Liquid Chromatography/Electrospray Ionization/Mass Spectrometry**—Lipid-soluble HDL oxidation products were extracted, as described previously, after addition of an internal standard (dipentadecanoyl glycerophosphocholine), the organic phase was evaporated under nitrogen, and lipids were redissolved in 500 μl of chloroform/methanol 2:1 (v/v). LC/ESI/MS analysis was performed using a normal phase silica column (4.6 × 250 mm, Alttech Associates, Deerfield, IL), in a Hewlett-Packard model 1050 liquid chromatograph, connected to a Hewlett-Packard model 5989A Quadrupole Mass Spectrometer (MS), equipped with a nebulizer-assisted electrospray ionization (ESI) interface (HP 59987A). The column was eluted with a linear gradient of 100% A (chloroform/methanol/80% ammonium hydroxide, 80:19.5:0.5 by volume) and 100% B (chloroform/methanol/water/30% ammonium hydroxide, 60:34:5.5:0.5 by volume) for 14 min, followed by 100% B for 10 min, at a flow rate of 1 ml/min (31). The effluent was split 1:50, resulting in 20 μl/min being admitted into the mass spectrometer. The retention time for phosphatidylcholine was determined using standard 16:0:20:4 glycerophosphocholine. The capillary exit voltage was set at 150 V, with electron multiplier at 1795 V. Positive ESI spectra were examined in the mass range 450–1100 atomic mass units. The masses given in the figures are nominal masses. The actual masses of the [M + 1]⁺ (positive ion mode) ions are 1 mass unit higher. The molecular species of the oxidation products were identified based on the molecular mass of the components as follows: PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; LysoPC, lysophosphatidylcholine; PC-OH, phosphatidylcholine hydroperoxides; isoPC, isoprostane glycerophosphocholines; Al2PC, phosphatidylcholine core aldehydes. LC/ESI/MS conditions were as described under “Experimental Procedures”. The profile is representative of three separate experiments.

**Tryptic Cleavage of HDL-bound Proteins**—Trypsin was dissolved in PBS, pH 7.4, and added to HDL at an enzyme to HDL protein ratio of 1:10. The mixture was incubated overnight at 37 °C. HDL was diluted to 1 mg/ml using PBS and subjected to oxidation with SIN-1. At specific times, 1 μl of the oxidation mixture was withdrawn, and the reaction was stopped by the addition of 100 μM BHT, followed by extraction of the HDL lipids.

**Preparation of HDL Lipid Suspensions**—HDL was extracted with chloroform/methanol (2:1, v/v) and the solvent removed under N₂. Buffer was added; the sample was vortexed, and the lipid suspension was sonicated (model 1200 bath sonicator, Branson Instruments) for 2 min. This preparation, referred to as HDL lipid suspension, was oxidized with SIN-1 at 37 °C for up to 6 h, under conditions similar to those used for HDL oxidation. Lipid-soluble oxidation products were extracted with chloroform/methanol 2:1 (v/v), and after addition of the internal standard, dipentadecanoyl glycerophosphocholine, specimens were prepared for LC/ESI/MS analysis.

**Preparation of ApoA-I Proteoliposomes**—HDL was isolated from serum taken from subjects fasted for 12–14 h, by ultracentrifugation.
Procedures.

LC/ESI/MS conditions were as described under "Experimental Procedures." The concentration of 100 mM BHT (final concentration, 100 mM DTPA, at a final amount of 150 μM DTPA) was dried under nitrogen and redissolved in 500 μl of chloroform/methanol for LC/ESI/MS analysis. The lipid extract was digested by trypsin overnight at 37 °C with 2 μg of trypsin Poros beads (Perspective Biosystems). The digested peptides were fractionated on a 7.5-cm (100 μm inner diameter) reverse phase-high pressure liquid chromatography using Supelcosil LC-8 (250 × 4.6 mm inner diameter, Supelco, Bellefonte, PA), in Hewlett-Packard model 1090 liquid chromatograph equipped with photodiode array detector (Waters 990). The UV absorbance detector was set to 280 nm. The column was eluted with a linear gradient of 10% A (0.1% trifluoroacetic acid/water) to 80% B (0.1% trifluoroacetic acid/acetonitrile) in 20 min, followed by 80% B for 5 min, at a flow rate 1 ml/min. Further confirmation of the purity of our PON-1 preparation was obtained by microcapillary electrospray LC/MS/MS. Briefly, the equivalent of 1 μg of PON-1 in 100 μl NH4HCO3, 1 mM CaCl2 buffer, pH 8.5, was digested by trypsin overnight at 37 °C with 2 μl of immobilized trypsin Poros beads (Perspective Biosystems). The digested peptides were fractionated on a 7.5-cm (100 μm inner diameter) reverse phase C18 capillary column, attached in-line to a Finnigan LCQ-Deca ion trap mass spectrometer. The entire digested sample was loaded as described between densities 1.063 and 1.21 g/ml, and exhaustively dialyzed in PBS, pH 7.4. ApoA-I was isolated by ion-exchange HPLC using an Aquapore AX-300 column (3 cm × 4.6 mm, Pierce) at a flow rate of 1 ml/min, using a gradient of 0.03 M Tris, pH 7.45, 50% isopropanol and 0.3 M Tris, pH 7.45, 50% acetonitrile, run over 15 min. 1-Palmitoyl-2-linoleoyl glycerophosphocholine dihydroperoxides were prepared by incubation of PLPC with 10 mM t-butyl-hydroperoxide at 37 °C for 1 h, at which time the reaction was terminated by addition of BHT to a final concentration of 100 μM (32). Proteoliposomes enriched in PLPC hydroperoxides were prepared with PLPC hydroperoxides/lyso phosphatidylcholine/apoA-I in the molar ratio of 57:170:1 (33), and were incubated for up to 6 h at 37 °C in the presence of 100 μM DTPA. Proteoliposomes to be used for oxidation experiments, including controls, were prepared by cholate dialysis of emulsions of apoA-I/PLPC/DMMC/cholesterol in a molar ratio of 57:170:1 (33), and were incubated for up to 6 h at 37 °C in the presence of 100 μM DTPA. Proteinoliposomes were incubated with 1 mM SIN-1 for up to 6 h at 37 °C in the presence of 100 μM DTPA, at a final amount of 150 μg of phosphatidylcholine. Liposomes (without protein) were also prepared and oxidized by SIN-1. At each time point, for all experiments, an aliquot was withdrawn; the oxidation was stopped by the addition of BHT (final concentration, 100 μM), and lipid-soluble oxidation products were extracted with chloroform/methanol 2:1 (v/v). The lipid extract was dried under nitrogen and redissolved in 500 μl of chloroform/methanol for LC/ESI/MS analysis.

Preparation of Core Aldehyde Proteoliposomes—Proteoliposomes were prepared by cholate dialysis (35). Briefly, apoA-I in 10 mM Tris buffered at pH 7.4 was dialyzed against 10 mM Tris-HCl, 0.15 M NaCl buffer, pH 7.4.

Serum PON-1 Purification—PON-1 Q192 type was purified from outdated human plasma through (pseudo) affinity chromatography using Cibacron Blue 3GA (Sigma) and anion-exchange chromatography consisting of two sizeexclusion DEAE-Bio-Gel A (Bio-Rad) columns as described (36, 37). SDS-PAGE analysis of purified PON-1 showed one broad band at 45 kDa corresponding to PON-1. In order to check for other residual protein contaminants such as PAF-AH or lecithin:cholesterol acyltransferase, we analyzed the PON-1 preparation by reverse phase-high pressure liquid chromatography using Supelcosil LC-8 (250 × 4.6 mm inner diameter, Supelco, Bellefonte, PA), in Hewlett-Packard model 1090 liquid chromatograph equipped with photodiode array detector (Waters 990). The UV absorbance detector was set to 280 nm. The column was eluted with a linear gradient of 10% A (0.1% trifluoroacetic acid/water) to 80% B (0.1% trifluoroacetic acid/acetonitrile) in 20 min, followed by 80% B for 5 min, at a flow rate 1 ml/min. Further confirmation of the purity of our PON-1 preparation was obtained by microcapillary electrospray LC/MS/MS. Briefly, the equivalent of 1 μg of PON-1 in 100 μl NH4HCO3, 1 mM CaCl2 buffer, pH 8.5, was digested by trypsin overnight at 37 °C with 2 μl of immobilized trypsin Poros beads (Perspective Biosystems). The digested peptides were fractionated on a 7.5-cm (100 μm inner diameter) reverse phase C18 capillary column, attached in-line to a Finnigan LCQ-Deca ion trap mass spectrometer. The entire digested sample was loaded as described.

HCl buffer, pH 7.4, was added to a dried lipid film containing equimolar quantities of 1-palmitoyl-2-(5-oxo)valeroyl-sn-glycero-3-phosphocholine and the corresponding carboxylic acid, 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphocholine (C5 Ald PC and C5 Acid PC) or 1-palmitoyl-2-(9-oxo)nonanoyl-sn-glycero-3-phosphocholine and the corresponding carboxylic acid 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine (C9 Ald PC and C9 Acid PC), with dimyrystoylphosphatidylcholine and free cholesterol in molar ratio 0.8:30:30:120:25. The dispersed liposomes were dialyzed overnight against 10 mM Tris-HCl, 0.15 mM NaCl buffer, pH 7.4.

FIG. 2. Mass spectra of mono- and dihydroperoxides, isoprostanes, and core aldehydes accumulated during 6 h of oxidation of HDL with SIN-1. A, full mass spectrum averaged over the elution times of the hydroperoxide and isoprostane glycerophosphocholine peaks (16.5–18 min) from oxidation of HDL. B, selected positive ion mass spectrum averaged over the elution time of the phosphatidylcholine core aldehyde peaks (19–21.5 min) from oxidation of HDL. m/z 790, phosphatidylcholine monohydroperoxide; m/z 846, phosphatidylcholine dihydroperoxide; m/z 830, 16:0-E_D2 isoprostanes glycerophosphocholine; m/z 858, 18:0-E_D2 isoprostanes glycerophosphocholine; m/z 832, 16:0-F2 isoprostanes glycerophosphocholine; m/z 860, 18:0-F2 isoprostanes glycerophosphocholine; m/z 650, 16:0–9:0 aldehyde glycerophosphocholine; m/z 678, 18:0–9:0 aldehyde glycerophosphocholine. LC/ESI/MS conditions were as described under "Experimental Procedures."
Results

Identification of Phosphatidylcholine Oxidation Products—Representative total positive ion current profiles for HDL, oxidized HDL, and oxidized HDL lipids are shown in Fig. 1. LC/ESI/MS analysis of HDL (Fig. 1A) showed phosphatidylcholine as the major component with smaller amounts of sphingomyelin and lysophosphatidylcholine, which are eluted in order of increasing polarity. An examination of the total ion current averaged over the phosphatidylcholine peak from HDL showed that the major components were 16:0/18:2 (m/z 758), 16:0/20:4 (m/z 782), 18:1/18:2 (m/z 784), 18:0/18:2 (m/z 786), and 18:0/20:4 (m/z 810) glycerophosphocholines. A minor peak was seen for the ethanolamine phospholipids, which are best determined in the negative ion mode. Following a 6-h incubation with SIN-1, the phosphatidylcholine peak was greatly reduced (Fig. 1B), whereas the peak for lysophosphatidylcholine was greatly increased. Peaks were seen with retention times corresponding to phosphatidylcholine hydroperoxides, isoprostanes, and core aldehydes. The mass spectrum, averaged over the lysophosphatidylcholine peak, indicated the presence of the 16:0 (m/z 494) and 18:0 (m/z 522) species as major components in both native and oxidized HDL. In contrast to HDL, phosphatidylcholine in the HDL lipid suspensions was much less affected by SIN-1 (Fig. 1C), as seen from the limited accumulation of phosphatidylcholine hydroperoxides, isoprostanes, core aldehydes, and lysophosphatidylcholine.

The mass spectra of the major components eluting from 16.5 to 18.0 min of a typical profile for HDL after 6 h of oxidation with SIN-1 is shown in Fig. 2. The major ions included m/z 790 (34:2 monohydroperoxy glycerophosphocholine); m/z 846 (36:4 dihydroperoxy glycerophosphocholine); m/z 830 (36:4 E/D isoprostanes), m/z 858 (38:4 E/D isoprostanes), m/z 832, (36:4 F isoprostanes), and m/z 860 (38:4 F isoprostanes) glycerophosphocholine (Fig. 2A). The major ions of a representative profile of HDL oxidized for 6 h and averaged over 19.0–21.5 min are shown in Fig. 2B. The ions m/z 594 (16:0/5:0), m/z 650 (16:0/9:0), and m/z 678 (18:0/9:0) represent the molecular ions of core aldehydes of glycerophosphocholines. There was also a small ion, m/z 622, representing the 18:0/5:0 core aldehyde glycerophosphocholine.
The major mono- and dihydroperoxy phosphatidylcholines present following a 2-h incubation of HDL with SIN-1 are shown in Fig. 3. The single positive ion mass chromatograms showed the m/z 814 (36:4) and m/z 816 (36:2) monohydroperoxides and the m/z 846 (36:4), m/z 848 (36:3), and m/z 874 (38:4) dihydroperoxide glycerophosphocholines. The monohydroperoxides eluted from the normal phase column before the dihydroperoxides, regardless of the length of the fatty acyl chains.

The major isoprostane derivatives of phosphatidylcholine present following a 2-h incubation of HDL with SIN-1 are shown in Fig. 4. Single positive ion mass chromatograms for the ions m/z 830 and 858 correspond to the 36:4 and 38:4 E2/D2 isoprostanes, whereas the ions m/z 832 and m/z 860 correspond to the 36:4 and 38:4 F2 isoprostanes. The ion m/z 828 corresponds to an isoprostane-like product, epoxy-isoprostane-oylglycerophosphocholine. The isoprostanes eluted as broad peaks, consistent with these compounds being a mixture of regioisomers. The 18:0/F2 isoprostane glycerophosphocholines were identified as homologues possessing 28 additional mass units.

The single positive ion mass chromatograms for the major core aldehydes of phosphatidylcholine that were present following a 2-h incubation of HDL with SIN-1 are shown in Fig. 5. The ions at m/z 594 and m/z 622 correspond to the 16:0/5:0 aldehyde and 18:0/5:0 aldehyde glycerophosphocholines, and ions at m/z 650 and m/z 678 correspond to the 16:0/9:0 aldehyde and 18:0/9:0 aldehyde glycerophosphocholines. The ions at m/z 636 and m/z 676 were attributed to the monohydroxy 16:0/9:1 aldehyde and the 18:1/9:0 aldehyde glycerophosphocholines, respectively. The aldehydes were eluted as sharp peaks in order of increasing polarity (decreasing chain length).

The accumulation of the hydroperoxides of phosphatidylcholine (Fig. 6A) was most rapid and reached the highest level during oxidation of native HDL, compared with HDL lipid suspensions or trypsinized HDL (p < 0.0013). In contrast, the accumulation of the isoprostane derivatives (Fig. 6B) was much more similar between native and trypsinized HDL and HDL lipid suspensions, although the initial rate of accumulation was highest for the native HDL (p < 0.035). The accumulation of core aldehydes (Fig. 6C) paralleled that of hydroperoxides and was higher for native HDL than trypsinized HDL or HDL lipid suspensions. The accumulation of the lysophosphatidylcholine (Fig. 6D) was highest for native HDL (p < 0.0001) compared with HDL lipid suspensions or trypsinized HDL.

Apolipoprotein A-I Promotes Formation of Phosphatidylcholine Core Aldehydes—ApoA-I proteoliposomes were incubated with SIN-1 for up to 6 h, and the formation of core aldehydes was measured. There was a significantly greater increase in PLPC hydroperoxides in the presence of apoA-I (Fig. 7, lower panel) parallel to the accumulation of core aldehydes compared with control (p < 0.0001) (Fig. 7, upper panel). The above experiments provided evidence that apoA-I promoted the formation of core aldehydes. However, to exclude the possibility that reaction between phosphatidylcholine oxidation products and SIN-1 may have confounded the observations, we studied the formation of core aldehydes in the absence of SIN-1 by using phosphatidylcholine hydroperoxides.

The effect of apoA-I on the conversion of phosphatidylcholine hydroperoxides into core aldehydes is shown in Fig. 8. Phosphatidylcholine hydroperoxides showed a modest decrease in the presence of apoA-I, compared with control incubations in the absence of apoA-I (Fig. 8, upper panel). There was a 2-fold higher concentration of core aldehydes in the presence of apoA-I, compared with the absence of apoA-I (Fig. 8, lower panel, p < 0.001).

PON-1 Has a Phospholipase A2-like Activity toward PC Core Aldehydes and Acids—ApoA-I proteoliposomes were prepared with DMPC and either a mixture of C5 Ald PC and C5 Acid PC or a mixture of C9 Ald PC and C9 Acid PC. PON-1 readily converted C5 Ald PC and C5 Acid PC proteoliposomes to lysophosphocholine (p < 0.0055 and p < 0.0001, respectively) (Fig. 9A) and also C9 Ald PC and C9 Acid PC proteoliposomes to lysophosphocholine (p < 0.001 and p < 0.0001, respectively) (Fig. 9B). The hydrolysis of C5 Ald PC was significantly greater than the hydrolysis of C9 Ald PC. C5 Acid PC and C9 Acid PC were hydrolyzed to a similar extent. Myristoyl lysoglycerophosphocholine was not detected, consistent with PON-1 being specific for short or intermediate length fatty acyl chains, but not the intact long chain fatty acids in the sn-2 position of PC. This is also consistent with the absence of the release of mono- or dihydroperoxynolone in the previous experiments (data not shown). The hydrolysis of the core aldehydes and acids by PON-1 in this experiment suggests that the relatively high
Concentrations of lysophosphatidylcholine during HDL oxidation by SIN-1 could be accounted for by PON-1.

**Effect of Oxidation on HDL Proteins**—The apoproteins of native and oxidized HDL were analyzed by SDS-PAGE and showed that monomeric apoA-I was the major component at all times. Bands with the molecular weight of the apoA-I dimer, apoA-I trimer, and apoA-I:apoA-II dimer were detectable by 1 h. The identity of these bands was confirmed by immunoblot with polyclonal anti-apoA-I antibodies (Fig. 10) and anti-apoA-II antibodies (data not shown).

**PON-1 Arylesterase Activity Is Reduced by Peroxidation**—HDL PON-1 arylesterase activity was relatively stable under control conditions (Fig. 11A, open circles). PON-1 arylesterase activity of HDL declined to about 60% of the original value within a few minutes of the addition of SIN-1 and decreased to 20% of the original value after 4 h, being maintained at this level for up to 20 h (Fig. 11A, open squares). To address whether the loss of activity was due to SIN-1-derived peroxynitrite or lipid oxidation products, the arylesterase activity of native HDL was followed during incubation with HDL lipid suspensions that had been oxidized by incubation with SIN-1 for 4 h (Fig. 11A, closed squares). This resulted in a significant decrease in arylesterase activity, although it was not as large as that observed by oxidation of HDL. This suggests that both peroxynitrite and lipid oxidation products are responsible for the large decrease in arylesterase activity.

**Loss of PON-1 Arylesterase Activity during PLPC Oxidation**—To characterize the loss of PON-1 arylesterase activity during oxidation of HDL with SIN-1, this activity was assayed over a period of 6 h during the oxidation of apoA-I proteoliposomes with SIN-1 and compared with its activity after incubation with SIN-1 or the PC oxidation products, C5 Ald PC or C9 Ald PC (Fig. 11B). PON-1 arylesterase activity was reduced by 30%, compared with control (p < 0.03) after 6 h when incubated...
with PLPC exposed to SIN-1. In contrast, when PON-1 was incubated for 6 h with C9 aldehyde- and C9 carboxylate PC or C5 aldehyde- and C5 carboxylate PC, there was minimal loss of PON-1 arylesterase activity (15 and 5%, respectively) compared with control. This demonstrates that PON-1 arylesterase activity is not inhibited by PC aldehydes. Incubation of PON-1 with 1 mM SIN-1 for 6 h reduced PON-1 arylesterase activity by 10%. Thus the marked loss of activity of PON-1 that we observed during oxidation of HDL was due to the additive effects of lipid oxidation products and SIN-1.

**DISCUSSION**

This study demonstrates that peroxynitrite is capable of oxidizing HDL lipids to a variety of products, the content and composition of which is modified by the presence of apoA-I and PON-1. The major phosphatidylcholine oxidation products were the mono- and dihydroperoxides, core aldehydes, and isoprostanes. The high yield of the phosphatidylcholine core aldehydes during peroxynitrite oxidation of native HDL, in the presence of metal chelators is of special interest, since this eliminates the potential confounding reactions of metal ions, such as Cu$^{2+}$ (40–42). Mashima et al. (13) showed that apoA-I was capable of stoichiometric conversion of phosphatidylcholine hydroperoxides to phosphatidylcholine hydroxides. The efficiency of this conversion was found to be low, compared with incubation of phosphatidylcholine hydroperoxides with whole plasma, but the conversion to core aldehydes was not studied. Our data suggest that the major product formed by the interaction of phosphatidylcholine hydroperoxides with apoA-I is the phosphatidylcholine core aldehydes. There may be differences in experimental conditions that explain the different conclusions of previous studies and the results presented here. However, none of the previous studies used LC/ESI/MS to study the intact phosphatidylcholine oxidation products, and thus they were not able to assess the formation of phosphatidylcholine core aldehydes.

Studies by Gardner et al. (43) have indicated that chemical decomposition of hydroperoxides to aldehydes requires alkaline conditions. However, Kanazawa and Ashida (44, 45) have recently reported that lipid hydroperoxides, liberated by intestinal lipases from triacylglycerols, decompose to aldehydes via epoxyketones and hydroxides. The exact mechanism of this reaction is unknown, and it is not known whether or not similar reactions take place with hydroperoxides and hydroxides attached to the fatty acids of glycerophospholipids. The observation that apoA-I can catalyze this decomposition suggests that specific amino acid(s) or a cluster of amino acids act as an active center in apoA-I that in the presence of lipid hydroperoxides enhances the formation of aldehydes.

The oxidation of methionine to methionine sulfoxide by lipid hydroperoxide is a two-electron oxidation (11, 12). The observation that both apoA-I and apoA-II can participate in this transformation suggests that the major requirement is for a methionine residue to be in the hydrophobic environment of the
oxidized HDL lipids (open squares) incubated with PLPC proteoliposomes alone (PON-1 arylesterase activity during oxidation of PLPC proteoliposomes in the course of the oxidation of native HDL, suggests the specific apoA-I tyrosine residues are involved in this reaction.  

Future studies will address whether specific apoA-I tyrosine residues are involved in this reaction.  

The high proportion of lysophosphatidylcholine, formed during the course of the oxidation of native HDL, suggests the presence of one or more phospholipase A$_2$-like enzymes. Since control incubations of HDL, in the absence of free radical-generating systems, yielded only minimal increases in lysophosphatidylcholine, it must be concluded that the(s) enzyme(s) became activated by the presence of oxidized phospholipids or are specific for oxidized phospholipids. The increased lysophosphatidylcholine content of oxidized LDL can be accounted for by PAF-AH (50, 51). HDL, however, contains much lower levels of PAF-AH than LDL, and MacPhee et al. (52) have shown that inhibition of PAF-AH by SB-222657, an azetidinone derivative, has little effect on lysophosphatidylcholine generation during HDL oxidation by Cu$^{2+}$. Our direct investigation of PON-1 demonstrates that it fulfills the criteria to be the phospholipase A$_2$ in HDL that is active during lipid oxidation. Our studies do not exclude the presence of additional enzymes. A second candidate is paraoxonase-3, which has been shown to be present in rabbit HDL (53). Paraoxonase-3 shares many of the same substrates as PON-1, and to date no reagent or experimental condition has been described to inhibit selectively PON-1 versus paraoxonase-3. Further studies are needed to determine whether additional enzymes are present in HDL that could also hydrolyze phospholipid oxidation products.  

The present study showed that incubation of HDL with SIN-1 led to an 80% reduction in the arylesterase activity of PON-1. This report is consistent with Aviram et al. (54) in which they showed PON-1 activity was inhibited by 31–65% (assayed as arylesterase) by PAPC or oxidized cholesterol-linoleate (55). PON-1 can hydrolyze multiple substrates with different catalytic activities (56, 57), including PAF-AH-like activity (58). Thus, although PON-1 arylesterase activity is inhibited during HDL oxidation, it appears that its phospholipase A$_2$-like activity toward core aldehydes is maintained.

We measured the phosphatidylcholine isoprostanes of the E$_2$/D$_2$ and F$_2$ series over a wide range of experimental conditions. The relatively similar yields of these products suggest that their formation is determined by the chemical reaction of peroxynitrite and arachidonic acid with little or no influence due to the proteins of HDL.

Remarkably, the present study has shown that apoA-I increases the yield of a class of oxidized phospholipids, the core aldehydes, that have been identified as a major bioactive component of minimally modified LDL (59). We propose that, in vivo, this function of apoA-I is coupled with the activity of PAF-AH and PON-1 to effectively divert phosphatidylcholine hydroperoxides to biologically inactive products. However, in the context of a pro-inflammatory response, apoA-I would maintain its function while the activity of both PAF-AH and PON-1 would decrease. This duality of function for HDL would be consistent with the observations and hypothesis of van Lenten et al. (18) that HDL can be converted from an anti-inflammatory lipoprotein to a pro-inflammatory lipoprotein (19).

In summary, the present results show that peroxynitrite oxidation of HDL phosphatidylcholine results in a broad spectrum of oxidation products. However, the proportions of the types of oxidized phosphatidylcholines and lysophosphatidylcholines are significantly affected by apoA-I and PON-1. The yields of the core aldehydes, which are generated by cleavage of the peroxidized fatty acyl chains, are probably underestimated as they are continuously degraded by the phospholipase A$_2$-like activity of the oxidized HDL.

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