Supplemental Electronic Information

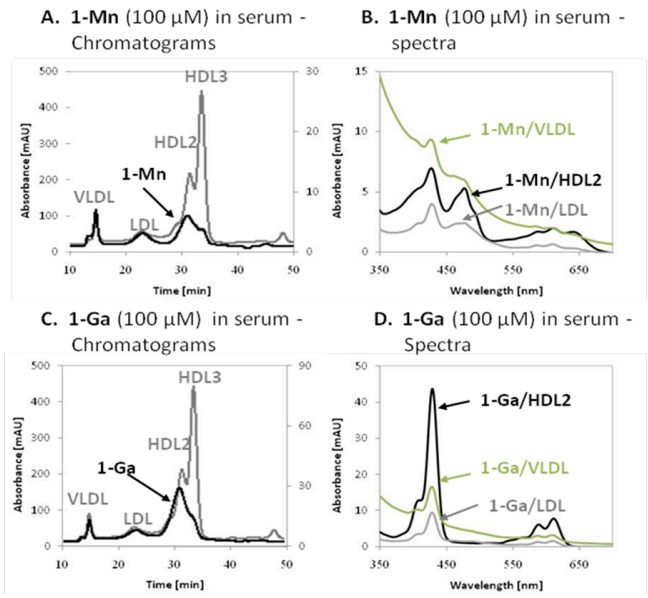

Fig S1 Serum distribution of **1-Mn** and **1-Ga**. The chromatograms at 280 nm (light line, relative to the left y-axis) and at 420 nm (dark line, relative to the right y-axis) obtained by HPLC separation of serum containing 100 µM **1-Mn** (**A**) or **1-Ga** (**C**). The absorbance spectra of corrole-HDL (dark line), corrole-LDL (light line) and corrole-VLDL (dotted line) conjugates eluted with **1-Mn** (**B**) or **1-Ga** (**D**).

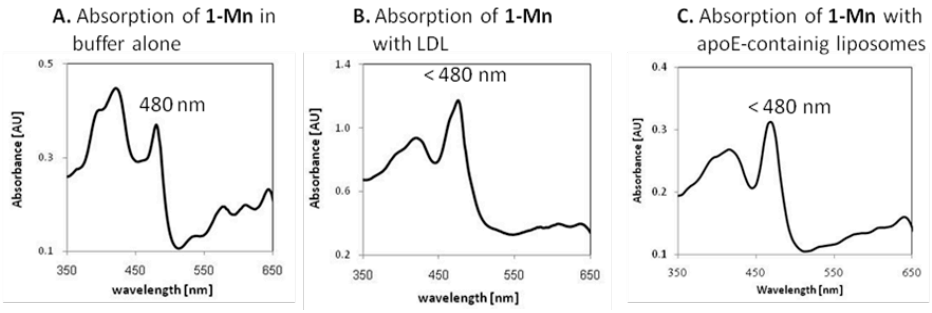

Fig. S2 Absorption spectrum of **1-Mn** in buffer **(A)**, with LDL **(B)** or with a very high histidine excess **(C)**.

Experimental section

Corrole preparation. Corroles were prepared according to previously described procedures [1,2], while the **2-Fe** was purchased from Frontier Scientific.

Lipoproteins preparation. Lipoproteins were separated from serum of normal healthy volunteers by sequential ultracentrifugation [3] and dialyzed against saline with EDTA (1 mM) for removing excess salt. Protein concentration of the separated fraction was determined by the lowry method [4]. Fraction purity was verified by HPLC injections as described later on. Oxidized LDL (oxLDL) was prepared by diluting LDL to 1 mg protein/ml and adding 40 μM $CuSO_4$ for several hours, until the yellow solution turned white.

Serum distribution of corroles

a) KBr-density gradient distribution of corroles. 40 μM of corrole/porphyrin was added to 4 mL of normal human serum and co-incubated for at least 10 minutes prior to further treatment. After 30 minutes of equilibration, the mixtures were treated for 48 h by ultracentrifugation in a KBr density gradient as previously described [3]. This treatment fractionates the serum to VLDL, LDL, HDL and LPDS appearing as yellowish rings, and the fraction location of the corroles or porphyrins is detected by their color (green and red, respectively).

b) HPLC distribution at low corrole concentrations. 100 μM of corrole/porphyrin was added to normal human serum and co-incubated for at least 10 minutes prior to further treatment. The solutions were filtered through a 0.22 μm filter and 50 μl were injected to a LaChrom Elite HPLC system fitted with a superose 6 10/300 GL (GE healthcare) gel filtration column and a photodiode array detector. The sample was eluted with PBS (pH = 7.4) at a flow rate of 0.5 ml/minutes. Chromatograms at 280 and 420 nm and the full spectrum of the run were recorded.

c) HPLC distribution for serum overloaded with 1-Fe. 1-Fe was added to the human serum in 1:1 volume ratio, giving a final corrole concentration of 500μM and two-fold diluted serum. These solutions were dialyzed against 3 replacements of saline-EDTA for removing all non-tightly bound corrole, and then filtered through a 0.22 μm and injected to the HPLC as described above.

d) 1-Fe distribution in the serum of knockout mice. Blood was collected from C57Bl/6 mice and from apoE and PON1 deficient mice (both on a background of C57Bl/6) on a regular diet. After 30 minutes the blood was centrifuged at 3000 rpm for 10 minutes, and the serum collected. Corroles were added to the mice serum to a final concentration of 100 μM, the solutions were filtered and 30 μl were injected to the HPLC as previously described.

Binding modes of corroles within lipoproteins

a) Interaction of corroles with amino acids esters. Concentrated solutions of MeHis, MeMet, MeTyr, EtCys, MeArg and MeLys were prepared. Microliters of these solutions were added to a 50 μM solution of corrole, and the electronic spectrum was measured. For recording the corrole spectrum with a very large excess of MeHis, the ester was dissolved in water in a very high concentration, the solution was brought to neutral pH, a droplet of corrole solution was added, and the absorbance measured.

b) Interaction of corroles with lipoproteins by CD spectroscopy. 60 μM of **1-Fe** or **1-Mn** or 15 μM of **1-Ga** were incubated for 10 minutes with 2.5 mg/ml HDL2 (~15 μM) or 2.5 mg/ml LDL (~5 μM), and then CD spectra at the visible range were measured on a JASCO J-810 CD spectropolarimeter using a data pitch of 1 nm, a bandwidth of 1 nm, a detector response of 1 sec and a scan speed of 100 nm/minutes.

c) Interaction of corroles with liposomes. Di-oleoyl-phosphatidylcholine, unesterified cholesterol and cholesterol oleate ester were each dissolved in a methamol:chloroform (1:2) solution, and mixed to a final concentration of 2.6 mM, 90 μM and 90 μM (in 1 ml), respectively (*5*). The solvent was evaporated under N_2, and 100 μl of tris-buffered saline (TBS: 20 mM tris, 150 mM NaCl, pH = 8) was added. A 1 minutes vortex followed by 2 minutes sonication and another 1 minutes vortex were applied, followed by 30 minutes shaking at 37 °C. For constructing lipidic liposomes, another 900 μl of TBS were added for 2 h incubation at 37 °C under shaking. PON1 or apoE were then added to a concentration of 0.2 μM, and another 2 h shaking at 37 °C was applied. Microliters of these solutions were added to a 30 μM solution of corrole, and the electronic spectrum was measured.

Attenuation of oxidative damage of LDL and HDL

a) LDL oxidation. 0.1 mg protein/ml of LDL in PBS was incubated with or without 2.5 μM of **1-Fe** for 10 minutes, and then 250 μM SIN-1 or 5 μM $CuSO_4$ were added for 2 hours incubation at 37 °C. At the end of oxidation solutions were analyzed for oxidation by measurement of conjugated dienes, hydroperoxides or aldehydes formation[6].

b) HDL oxidation. 1 mg protein/ml of HDL in PBS was incubated with or without 50 μM of **1-Fe** for 10 minutes (until **1-Fe** containing solutions turned green), and then 500 μM SIN-1 or 40 μM $CuSO_4$ were added for overnight incubation at 37 °C. At the end of oxidation solutions were analyzed for oxidation by measurement of conjugated dienes, hydroperoxides or aldehydes formation[6], as well as for tyrosine nitration. Tyrosine nitration was detected by western blot analysis using 20 μl of each HDL solution on a 12% SDS-PAGE. Blocking was obtained with 2% BSA overnight at 4 °C, followed by treatment with a biotinylated anti-nitrotyrosine monoclonal antibody (1:200, Cayman Chemicals) for 2 h, and then a peroxidase-streptavidine conjugate (1:10,000, Jackson ImmunoResearch Laboratories Inc.)

for 1 h, each in 1 % BSA at room temperature. The membrane was developed using an EZ-ECL kit.

Atherogenicity of corrole-treated lipoproteins

a) Corroles effect on HDL cholesterol efflux from macrophage. J774A.1 cells ($1x10^6$/mL) were incubated for 1 hour in serum-free DMEM that contained ^{3}H-cholesterol (2 μCi/mL) and BSA (0.2%). Cells were washed and then incubated in 1 mL of DMEM containing 100 μg of the various HDL solutions. After 4 hours incubation at 37 °C, 500 μL of the medium was collected. The cells were washed with PBS, 1 mL of 0.1 N NaOH was added and 500 μL was collected the next day. Medium and cellular ^{3}H-cholesterol were determined by liquid scintillation counting. The percentage of cholesterol efflux was calculated as the ratio of total counts per minute in the medium divided by the total counts per minute in the medium and in the cells. HDL-mediated cholesterol efflux was calculated after subtraction of the non-specific efflux obtained in cells incubated in the absence of HDL.

b) Corroles effect on HDL attenuation of macrophage oxidative stress. J774A.1 cells ($1x10^6$/mL) were treated with 50 μg protein/ml of various HDL solutions in serum free media. After 30 minutes incubation at 37 °C, oxLDL was added at a 25 μg protein/ml concentration for 4 hours, after which cells were washed and cellular oxidative stress was measured using the 2',7'-dichlorofluorescein diacetate (DCFH-DA) assay[7]. 10 μM DCFH-DA was added for 1 hour at 37 °C, and then the cells were washed and gently scraping into PBS. Cellular fluorescence was measured by flow cytometry with excitation at 488 nm and measurement of emission at 510-540 nm. oxLDL-induced oxidative stress was calculated by subtracting the basal cellular oxidative stress (without oxLDL and without HDL) from each recorded value.

c) Corroles effect on HDL attenuation of macrophage apoptosis. J774A.1 cells ($1x10^6$/mL) were treated with 100 μg protein/ml of various HDL solutions in serum free media. After 30 minutes incubation at 37 °C, tunicamycin was added at a 1 μg/ml concentration overnight, after which cells were washed and cellular viability was measured using the 3,3'-dihexyloxacarbocyanine iodide ($DiCO_6$) assay[8]. 50 μM $DiCO_6$ was added for 1 hour at 37 °C, and then the cells were washed and gently scraping into PBS. Cellular fluorescence was measured by flow cytometry with excitation at 488 nm and measurement of emission at 510-540 nm. Tunicamycin-induced apoptosis was calculated by subtracting recorded values from the value of untreated cells (without tunicamycin and without HDL).

d) Corroles effect on oxLDL induced oxidative stress in macrophage. 1 mg protein/ml oxLDL was incubated overnight at 37 °C in the presence or absence of 10 μM **1-Fe**. These oxLDL solutions were added at a 25 μg protein/ml concentration for 4 hours to J774A.1 cells ($1x10^6$/mL) in serum free media. Then the cells were washed and cellular

oxidative stress was measured using the 2',7'-dichlorofluorescein diacetate (DCFH-DA) assay[7]. oxLDL-induced oxidative stress was calculated by subtracting the basal cellular oxidative stress (without oxLDL)

Statistical analysis.

Each separate experiment was performed in triplicate, and each individual experiment was replicated 3 times (n=3). Statistical analyses were performed using Student's t test for comparing differences between the 2 groups.

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