Supplemental Material

Electrospray Tandem Mass Spectrometry Reveals Extensive and Non-specific Oxidation of Cholesterol Esters in Human Atheromata

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Supplementary Figure 1. NP-HPLC-MS/MS Calibration curves of commercially available CEs and oxCEs. Data points represent peak ratios when varying amounts of commercially available CEs and oxCEs analyzed with 0.5 ng of the internal standard: cholesteryl 9Z-heptadecenoate (17:1-CE). This was the same amount of internal standard spiked into biological extracts of human atheromata. Peak areas, peak area ratios and linear regressions were determined using Analyst MultiQuant software. The slope of each regression and its r^2 value is tabulated in the inset.

Supplementary Figure 2. Simultaneous NP-HPLC-MS/MS analysis of endogenous and isotopically labeled oxCEs following co-homogenization and extraction in the presence of cholesteryl 17,17,18,18-d_4-linoleate. All traces are extracted ion chromatograms using precursors of *m/z* 369.3 (A) The positive axis shows extracted ion chromatograms of the naturally occurring 18:2-CE and its must abundant oxidation product, HODE-CE, following homogenization and extraction from a human femoral endarterectomy atheromata. (B) The negative axis shows extracted ion chromatograms of d_4-cholesteryl linoleate and d_4-HODE-CE, which were not detected after co-homogenization and extraction. All chromatographic peaks are labeled with the cholesteryl ester species and the *m/z* extracted.

Supplemental Figure 3. NP-HPLC-MS/MS analyses of oxCE species containing hydroxyl moieties, before and after trimethylsilylation with BSTFA. All traces show extracted ion chromatograms from precursors of *m/z* 369.3 data. Various families of oxCE were isolated from human atheromata by preparative NP-HPLC-MS/MS and subjected to derivatization. Aliquots of (A) HODE-CEs, (B) HETE-CEs, (C) HpODE-CEs, (D) HpETE-CEs, and (E) hydroxy-epoxy-linoleate-CEs where taken for analysis before and after derivatization. Blue traces indicate endogenous oxCEs analyzed prior to derivatization, red traces indicate the trimethylsilylated species observed after BSTFA treatment (+72 amu). (E,F) Green chromatograms represent doubly-trimethylsilylated products - none were detected.

Supplemental Figure 4. NP-HPLC-MS/MS analyses of oxCE species containing ketone or aldehyde moieties before and after conversion to methoximes by gaseous methoxylamine treatment. All traces show extracted ion chromatograms from precursors of *m/z* 369.3 data. Various families of oxCE were isolated from human atheromata by preparative NP-HPLC-MS/MS and subjected to derivatization. Aliquots of (A) oxoODE-CEs, (B) keto-epoxy-linoleate-CEs and (C) 9-oxononanoate-CE where taken for analysis before and after derivatization. Blue traces indicate endogenous oxCEs analyzed prior to derivatization, red traces indicate the methoxime species formed during methoxylamine treatment (+29 amu).

Supplemental Figure 5. NP-HPLC-MS/MS analysis of triacylglycerides extracted from human atheromata (atheroma sample *iii*, Figure 5). (A) Full scan total ion chromatogram showing the elution of TAG species from 14-17 min. (B) Integrated spectrum summed over the TAG elution window. Peaks are labeled with *m/z* and TAGs are identified by the total acyl chain carbons and total double bonds. (C) Extracted ion chromatograms of major TAG and theoretical oxTAGs. Red traces: extracted ion chromatograms of major TAGs, $[M+NH_4]^+$, labeled red in panel C. Blue traces:

extracted ion chromatograms of theoretical oxidation products of the same major TAG species (plus a single oxygen, +16 amu), $[M+O+NH_4]^+$.

Supplemental Figure 6. NP-HPLC-MS/MS analysis of triacylglycerides extracted from human atheromata before and after ozone treatment. Blue trace: full scan total ion chromatogram of human plaque extract. Red trace: full scan total ion chromatogram of human plaque extract following ozone treatment. Peaks representing unmodified TAGs and oxidized TAGs are indicated. Oxidized TAGs, mainly chain-shortened ozonolysis products, were further characterized by MS/MS.

Supplementary Table 1: Mass transitions used during multiple reaction monitoring NP-HPLC-MS/MS experiments.

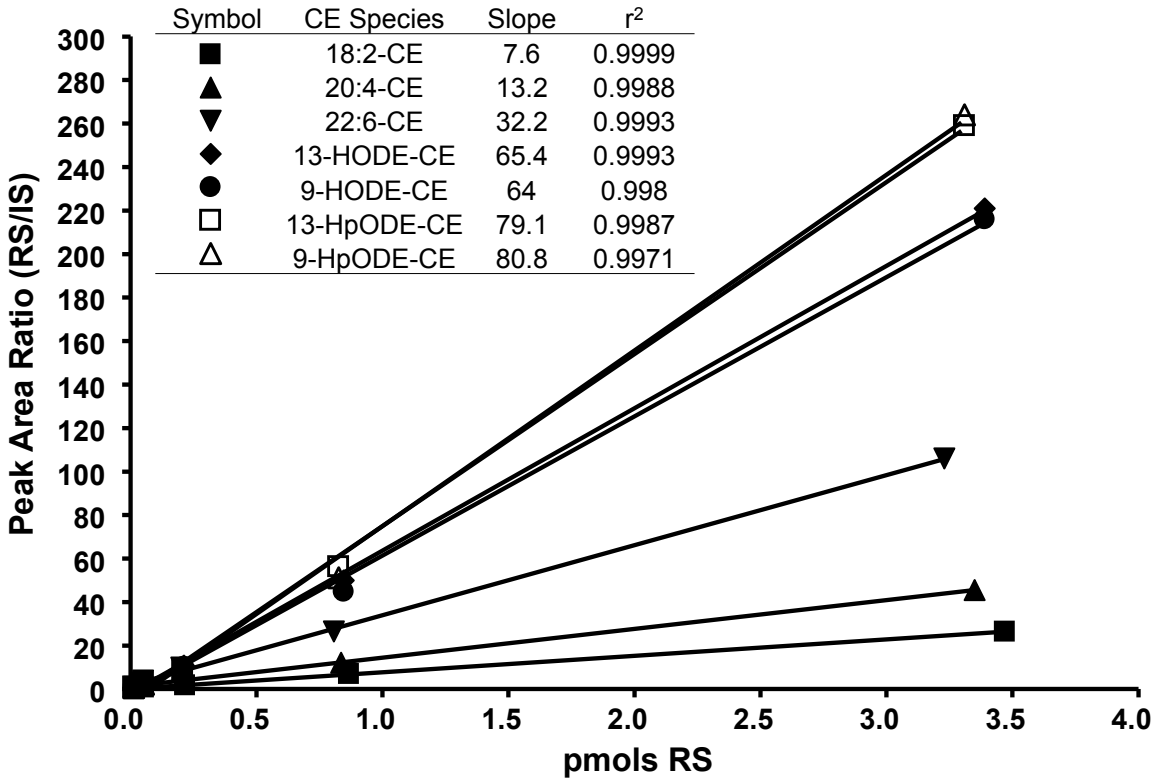
Cholesteryl Esters and Oxidation Products [a]	Ion Species	Q1 *m/z* [b]	Q3 *m/z* [c]
18:2	$[M+NH_4]^+$	666.6	369.3
20:4	$[M+NH_4]^+$	690.6	369.3
22:6	$[M+NH_4]^+$	714.6	369.3
17:1	$[M+NH_4]^+$	654.6	369.3
18:2 + O - H_2	$[M+H]^+$	663.6	369.3
18:2 + O - H_2	$[M+NH_4]^+$	680.6	369.3
18:2 + O	$[M+NH_4]^+$	682.6	369.3
18:2 + O_2 - H_2	$[M+NH_4]^+$	696.6	369.3
18:2 + O_2	$[M+NH_4]^+$	698.6	369.3
20:4 + O - H_2	$[M+H]^+$	687.6	369.3
20:4 + O - H_2	$[M+NH_4]^+$	704.6	369.3
20:4 + O	$[M+NH_4]^+$	706.6	369.3
20:4 + O_2 - H_2	$[M+NH_4]^+$	720.6	369.3
20:4 + O_2	$[M+NH_4]^+$	722.6	369.3
22:6 + O - H_2	$[M+H]^+$	711.6	369.3
22:6 + O - H_2	$[M+NH_4]^+$	728.6	369.3
22:6 + O	$[M+NH_4]^+$	730.6	369.3
22:6 + O_2 - H_2	$[M+NH_4]^+$	744.6	369.3
22:6 + O_2	$[M+NH_4]^+$	746.6	369.3

[a] Acyl chain components are denoted by number of carbons:double bonds; oxidative modifications are denoted by the addition or loss of the indicated species.

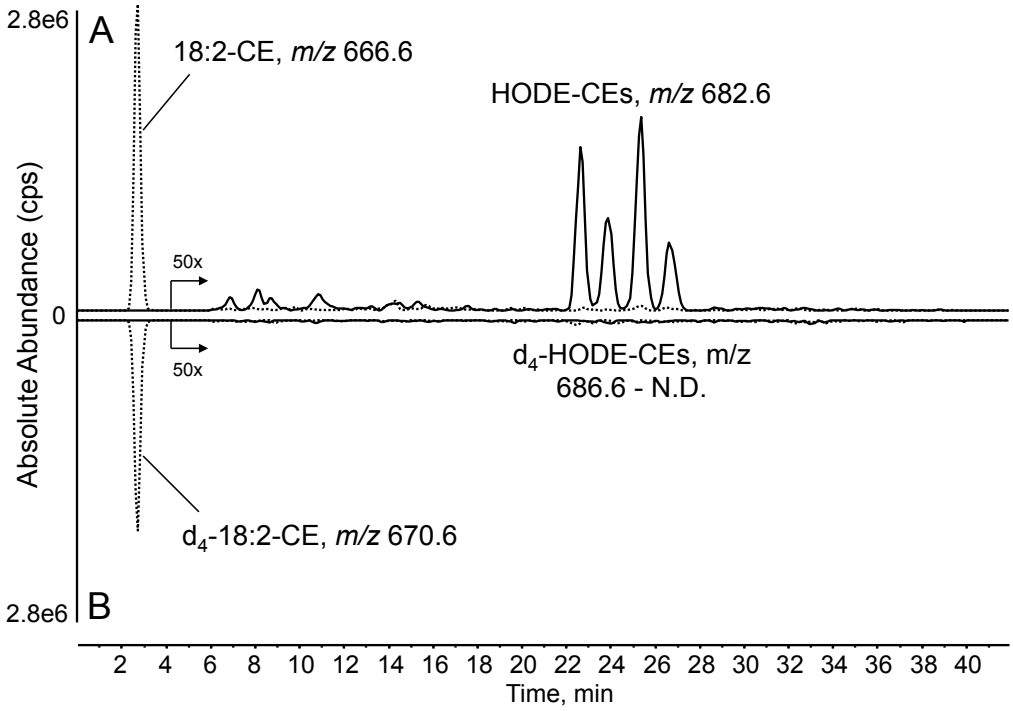
[b] *m/z* selected in the first quadrupole

[c] *m/z* selected in the third quadrupole

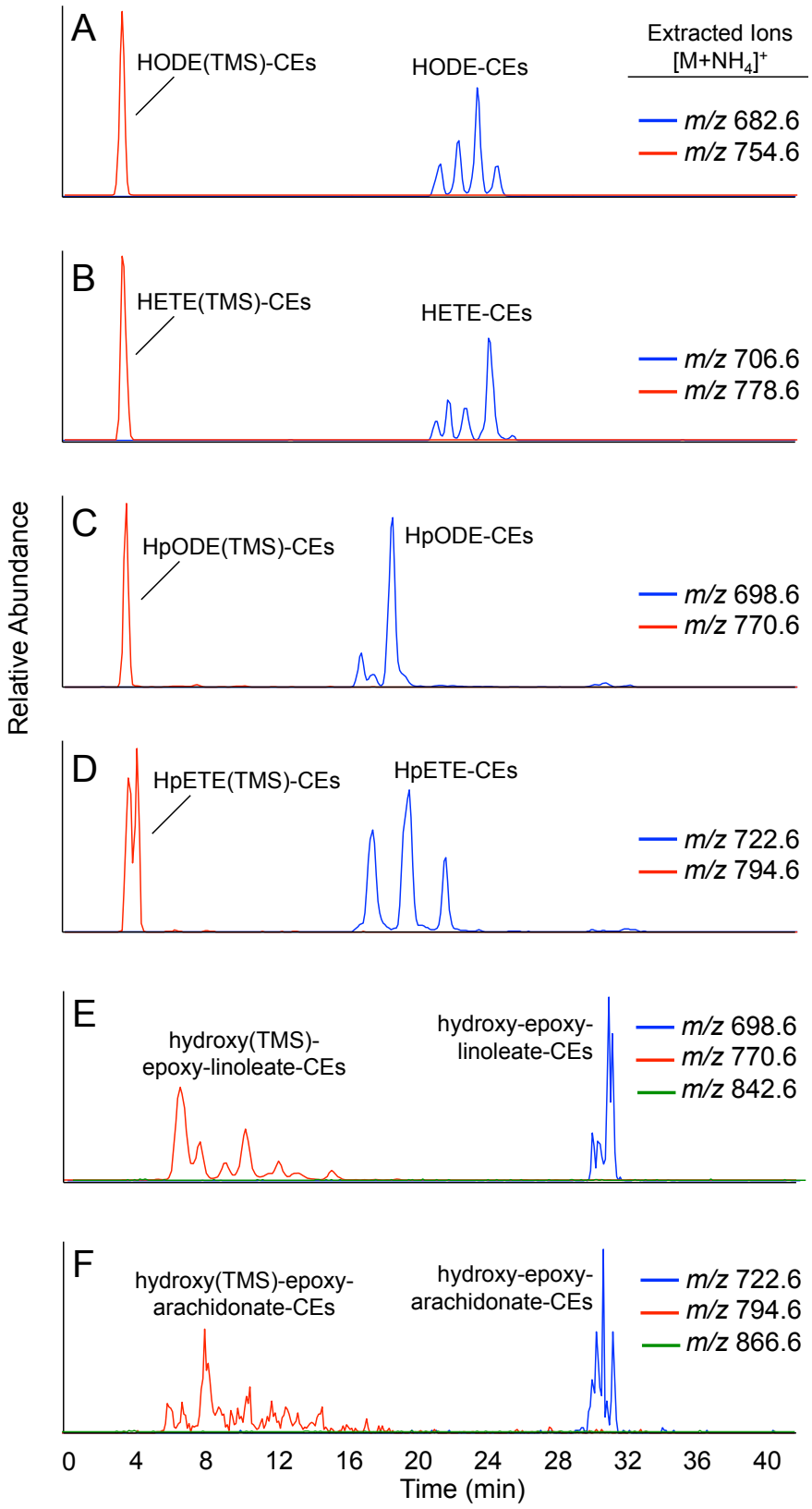
Supplemental Figure 1



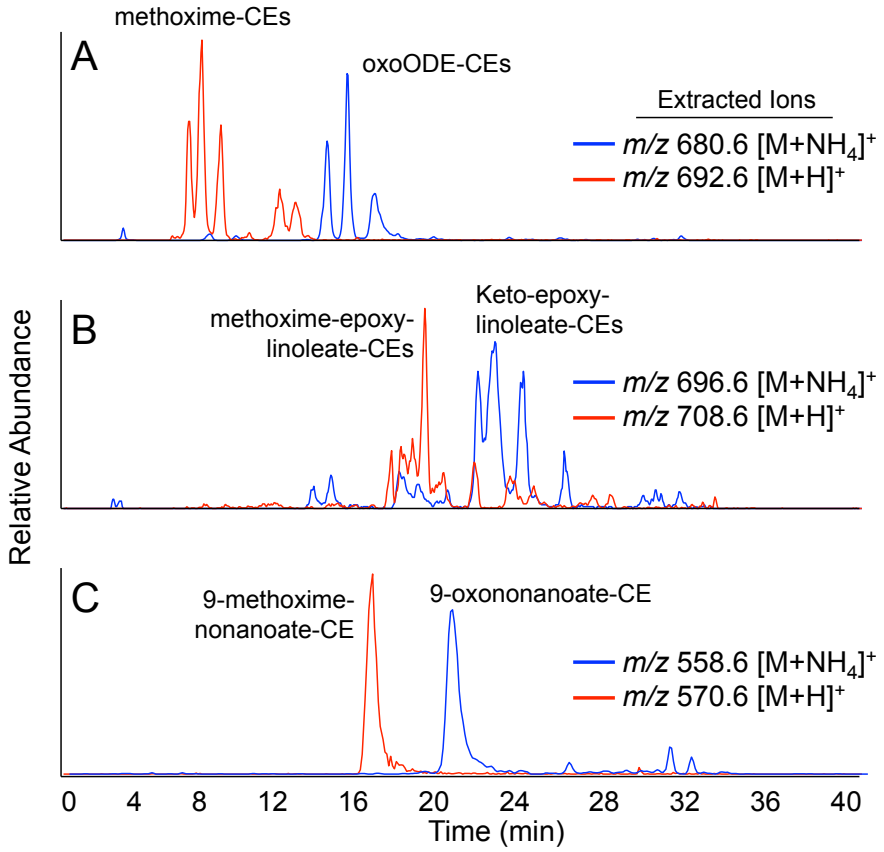
Supplemental Figure 2



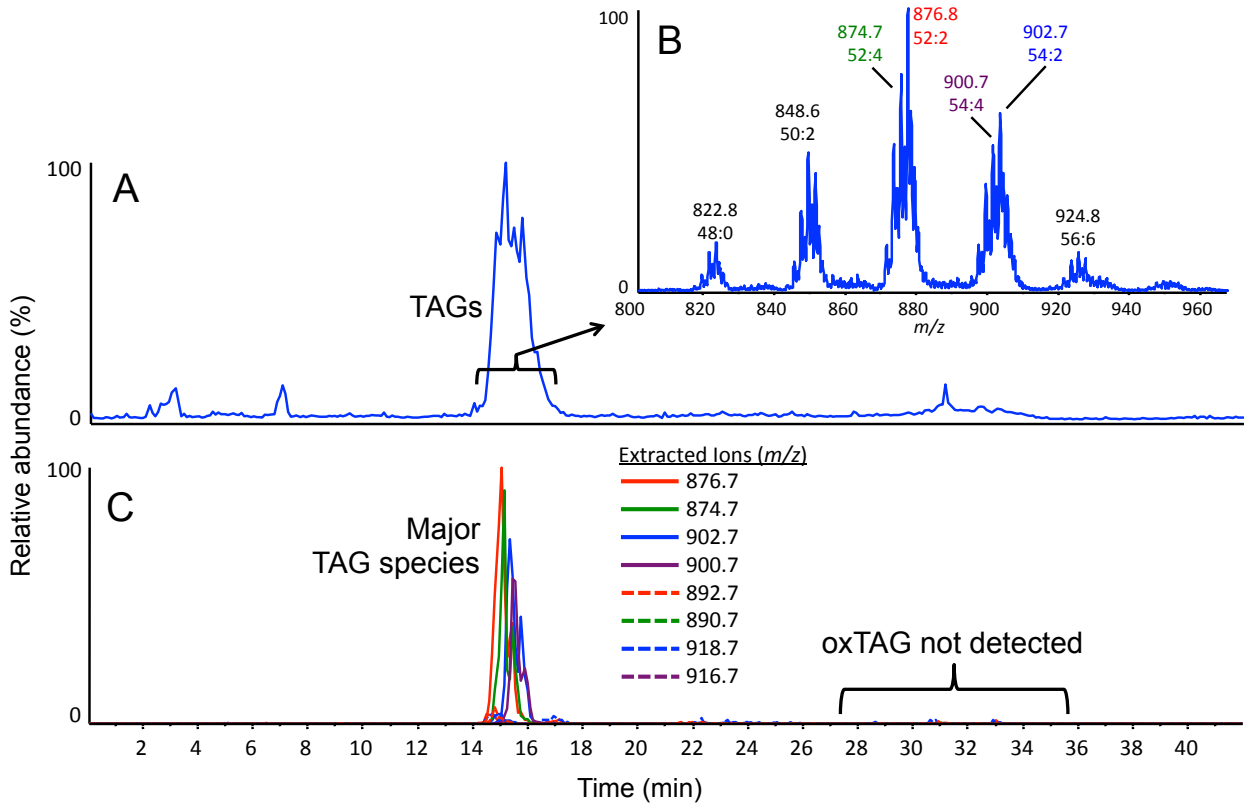
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6