Supplemental Material

The function and distribution of apolipoprotein A1 in the artery wall are markedly distinct from those in plasma

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Supplemental Table 1: Peak Lists of Apolipoprotein A1 Peptides in Lower ~25 kDa band verified by MS/MS

~25 kDa MW Lower Band Protein Identification

apolipoprotein A1 preproprotein **Sequence coverage 52%**

Observed Peptide Sequence	XCorr	Charge	m/z [Da]	ΔM [ppm]
AELQEGAR	1.82	2	437.2241	-1.85
ARAHVDALR	2.13	3	336.8608	-1.99
ATEHLSTLSEK	3.56	3	405.8789	0.46
DLATVYVDVLK	3.51	2	618.348	0.43
DYVSQFEGSALGK	4.16	2	700.8381	-0.30
EQLGPVTQEFWDNLEK	4.86	2	966.9692	-1.45
LEALKENGGAR	2.18	3	386.5468	-1.21
LLDNWDSVTSTFSK	5.09	2	806.8943	-2.54
QGLLPVLESFK	2.25	2	615.8572	-1.81
VQPYLDDFQK	2.00	2	626.8133	-1.25

ApoA1 was immuno-precipitated from normal artery wall homogenates and run on SDS-PAGE as described. The lower band migrating at ~25 kDa was excised (Supplemental Figure 2), digested with trypsin, and analyzed via nanospray-ESI on an Orbitrap-LTQ Velos. The resultant spectra were searched using Proteome Discoverer 1.1 against the Uniprot human database 2013_2. Shown is a listing of tryptic peptides confirmed by MS/MS for apoA1 with their respective SEQUEST correlation scores (XCorr), charge state, precursor mass and mass shift of the observed peptide from the theoretical mass (ΔM [ppm]).

Supplemental Table 2: Peak Lists of Apolipoprotein A1 Peptides in Upper~25 kDa band verified by MS/MS

~25 kDa MW Upper Band Protein Identification

apolipoprotein A1 preproprotein **Sequence coverage 44%**

Observed Peptide Sequence	XCorr	Charge	m/z [Da]	ΔM [ppm]
AKPALEDLR	3.25	3	338.19705	-2.00
ATEHLSTLSEK	3.49	3	405.87778	-2.32
DLATVYVDVLK	3.54	2	618.34631	-2.33
DYVSQFEGSALGK	4.64	2	700.83600	-3.26
EQLGPVTQEFWDNLEK	3.64	2	966.96796	-2.71
LLDNWDSVTSTFSK	4.75	2	806.89410	-2.77
QGLLPVLESFK	2.58	2	615.85687	-2.31
VQPYLDDFQK	2.39	2	626.81274	-2.12
VSFLSALEEYTK	5.02	2	693.85925	-2.84

ApoA1 was immuno-precipitated from normal artery wall homogenates and run on SDS-PAGE as described. The upper band migrating at ~25 kDa was excised (Supplemental Figure 2), digested with trypsin, and analyzed via nanospray-ESI on an Orbitrap-LTQ Velos. The resultant spectra were searched using Proteome Discoverer 1.1 against the Uniprot human database 2013_2. Shown is a listing of tryptic peptides confirmed by MS/MS for apoA1 with their respective SEQUEST correlation scores (XCorr), charge state, precursor mass and mass shift of the observed peptide from the theoretical mass (ΔM [ppm]).

Supplemental Table 3: Peak Lists of Apolipoprotein A1 Peptides in Lower ~50 kDa band verified by MS/MS

~50 kDa MW Lower Band Protein Identification

apolipoprotein A1 preproprotein **Sequence coverage 54%**

Observed Peptide Sequence	XCorr	Charge	m/z [Da]	ΔM [ppm]
AKPALEDLR	3.37	3	338.19766	-0.20
AKPALEDLRQGLLPVLESFKVSFLSALEEYTK	4.06	5	719.19965	0.47
ATEHLSTLSEK	3.18	3	405.87866	-0.14
DLATVYVDVLK	3.36	2	618.34692	-1.34
DSGRDYVSQFEGSALGK	5.47	3	605.95453	-1.01
DYVSQFEGSALGK	3.71	2	700.83752	-1.08
LEALKENGGAR	2.20	3	386.54700	-0.81
LLDNWDSVTSTFSK	4.20	2	806.89502	-1.63
LREQLGPVTQEFWDNLEK	6.07	3	734.70935	-2.53
LREQLGPVTQEFWDNLEKETEGLR	6.30	4	722.62006	-0.07
QGLLPVLESFK	2.70	2	615.85760	-1.12
VKDLATVYVDVLK	4.27	3	488.28836	-0.75
VQPYLDDFQK	2.50	2	626.81378	-0.47
VSFLSALEEYTK	4.70	2	693.86029	-1.35

ApoA1 was immuno-precipitated from normal artery wall homogenates and run on SDS-PAGE as described. The lower band migrating at ~50 kDa was excised (Supplemental Figure 2), digested with trypsin, and analyzed via nanospray-ESI on an Orbitrap-LTQ Velos. The resultant spectra were searched using Proteome Discoverer 1.1 against the Uniprot human database 2013_2. Shown is a listing of tryptic peptides confirmed by MS/MS for apoA1 with their respective SEQUEST correlation scores (XCorr), charge state, precursor mass and mass shift of the observed peptide from the theoretical mass (ΔM [ppm]).

Supplemental Table 4: Peak Lists of Apolipoprotein A1 Peptides in Upper ~50 kDa band verified by MS/MS

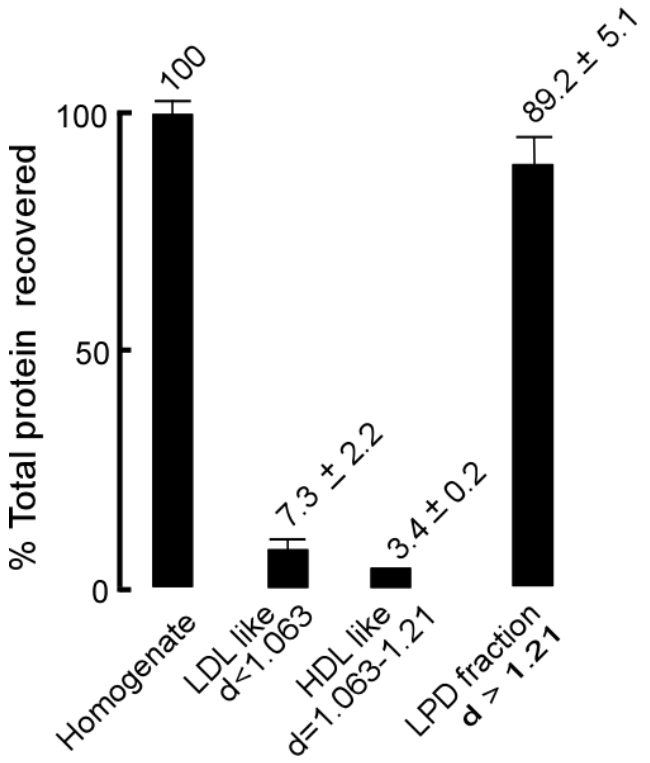
~50 kDa MW Upper Band Protein Identification

apolipoprotein A1 preproprotein **Sequence coverage 44%**

Observed Peptide Sequence	XCorr	Charge	m/z [Da]	ΔM [ppm]
AKPALEDLR	2.60	3	338.19690	-2.46
ATEHLSTLSEK	2.58	3	405.87784	-2.17
DLATVYVDVLK	3.22	2	618.34631	-2.04
DSGRDYVSQFEGSALGK	2.60	3	605.95453	-1.01
LLDNWDSVTSTFSK	2.58	2	806.89478	-1.93
LSPLGEEMR	3.22	2	516.26184	-2.58
QGLLPVLESFK	2.57	2	615.85699	-2.11
THLAPYSDELR	3.17	3	434.55347	-2.02
VKDLATVYVDVLK	2.97	3	488.28790	-1.62
VSFLSALEEYTK	4.33	2	693.85999	-1.79

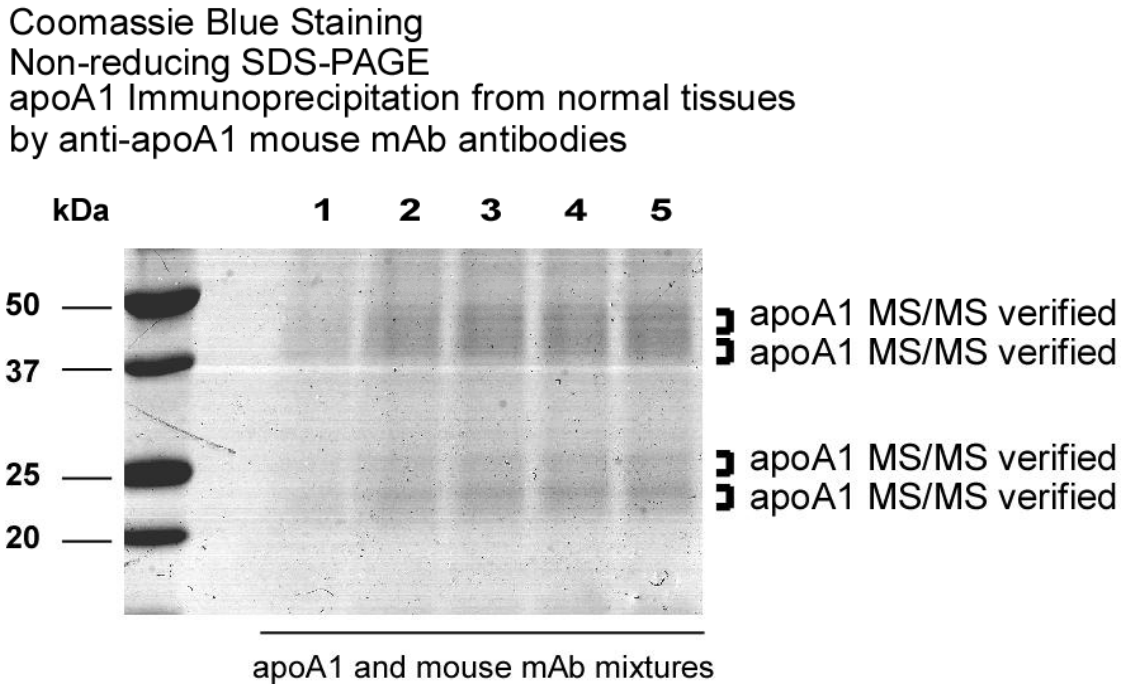
ApoA1 was immuno-precipitated from normal artery wall homogenates and run on SDS-PAGE as described. The upper band migrating at ~50 kDa was excised (Supplemental Figure 2), digested with trypsin, and analyzed via nanospray-ESI on an Orbitrap-LTQ Velos. The resultant spectra were searched using Proteome Discoverer 1.1 against the Uniprot human database 2013_2. Shown is a listing of tryptic peptides confirmed by MS/MS for apoA1 with their respective SEQUEST correlation scores (XCorr), charge state, precursor mass and mass shift of the observed peptide from the theoretical mass (ΔM [ppm]).

Supplemental Figure 1



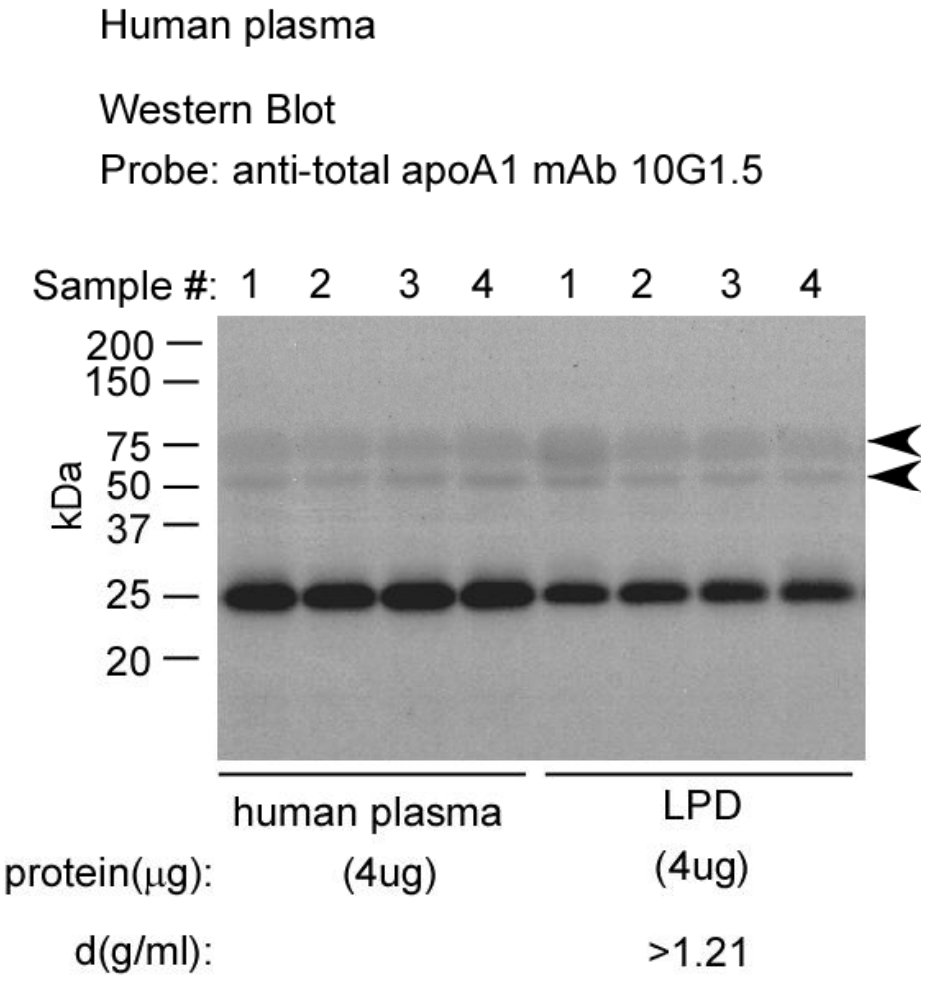
Supplemental Figure 1. The distribution of protein recovered in normal artery wall homogenate before (100%) and following fractionation by ultracentrifugation. Normal artery wall homogenates (n=5) were fractionated by sequential buoyant density ultracentrifugation using D_2O/Sucrose mixtures to produce the indicated density fractions, as described under Methods, and then total protein was determined from starting material and LDL-like, HDL-like and lipoprotein-depleted (LPD) fractions. The percentage of total protein relative to homogenate (100%) in each sub-fraction is indicated. Error bars indicate standard deviation. Note that the majority of protein recovered from normal artery wall homogenate is within the lipoprotein-depleted fraction.

Supplemental Figure 2



Supplemental Figure 2. ApoA1 is present in the normal artery wall and is cross-linked. ApoA1 was immuno-precipitated from normal artery wall homogenates (n=5) and separated on a non-reducing (5-15%) SDS-PAGE gradient gel and stained with Coomassie Blue. Indicated bracketed bands (upper and lower ~25 kDa and ~50 kDa protein bands) were excised from the gel and subjected to trypsin digestion and mass spectrometry analysis. The major peptides recovered and sequenced from each indicated excised regions of the gel were observed by LC-MS/MS to be from apoA1 (Supplemental Tables 1-4).

Supplemental Figure 3



Supplemental Figure 3. Plasma contains both monomeric (major) and slower migrating (minor) oxidized and cross-linked apoA1 forms. Indicated amounts of plasma and LPD fraction proteins (n=4) from those plasma samples were run on a reducing 12% SDS-PAGE gel. The proteins were then transferred to membrane for Western blot probing with mAb 10G1.5. The major bands noted within both plasma and the LPD fractions appear at the anticipated molecular weight of the apoA1 monomer (~25 kDa). Longer exposures (shown) of the Western blot reveal the presence of slower migrating apoA1 immuno-reactive bands (indicated by arrowheads), consistent with oxidatively cross-linked forms of apoA1.