

A Comparison of Methods to Enhance Protein Detection of Lipoproteins by Mass Spectrometry

Supporting Information

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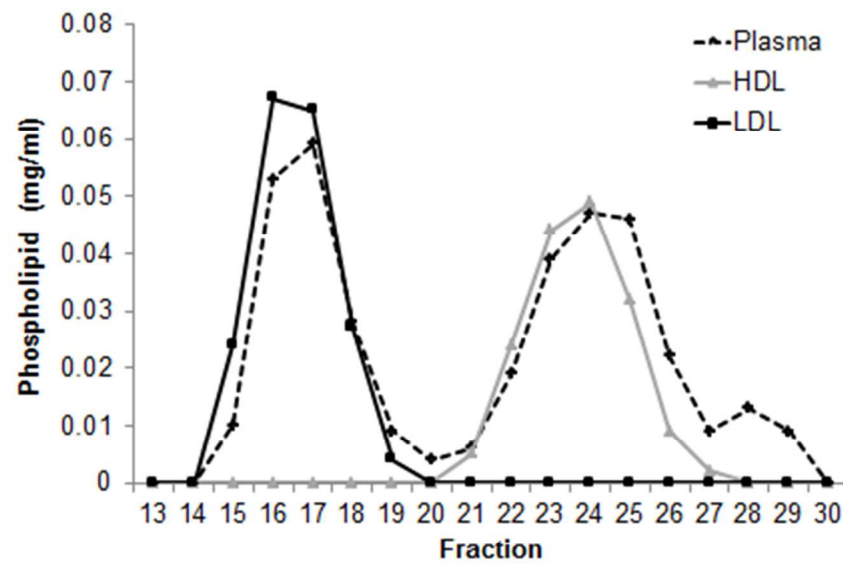
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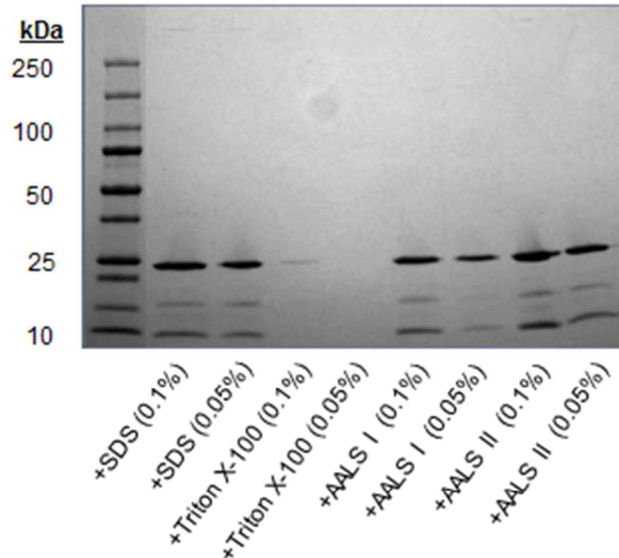
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Supplement Figure 1:



Phospholipid content in fractions separated by gel-filtration of plasma vs. HDL and LDL separated by ultracentrifugation from a single subject.

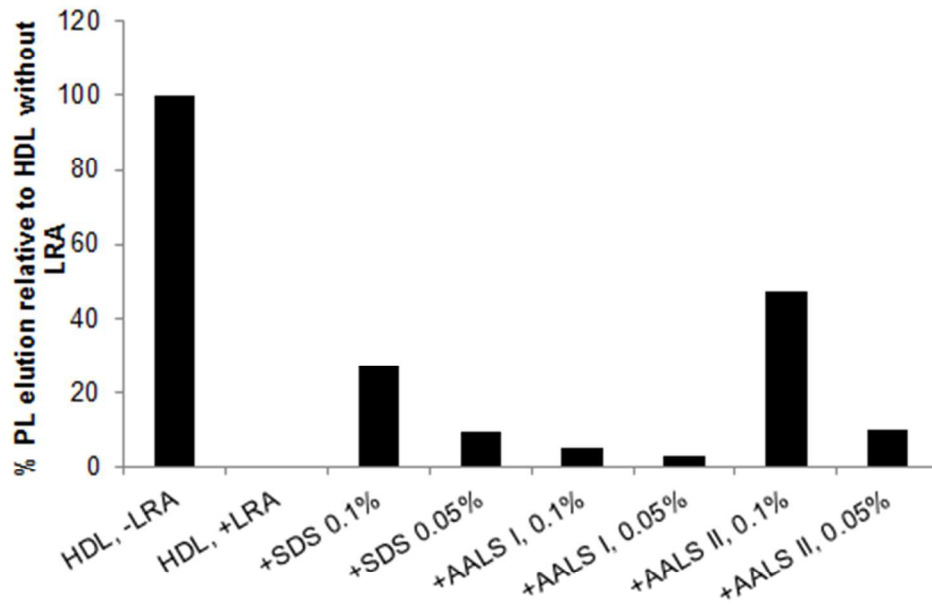
Supplement Figure 2:



Comparisons of protein elutions from the LRA with various detergents. SDS PAGE (4-15%)

comparison of UC-HDL protein elutions from LRA which were treated with the detergents SDS, Triton X-100, AALS I, or AALS II. 3.15 mg LRA from an LRA stock solution of 100 mg/ml was added to each experimental well of a 0.45 μ m filter plate (Millipore). 31.5 μ g of PL mass from UC isolated HDL in 50 mM AB buffer was then added and adjusted to a total volume of 50 μ l. The plate was then incubated for 30 minutes at RT. Flow-through was collected and the resin was washed three times with 150 μ l AB Buffer. Each detergent at concentrations of 0.1% and 0.05% was then added to each well in a total volume of 150 μ l AB Buffer and incubated for 30 minutes at RT. Samples were eluted from the LRA and the resin was washed twice more with 50 μ l AB Buffer. Equal volumes of 20 μ l from each sample were loaded onto the gel. The gel was stained with coomassie blue.

Supplement Figure 3:



Relative determination of phospholipid co-eluting with proteins off of the LRA. UC-HDL was exchange-labeled with 0.5% rhodamine-phospholipid (AVANTI). Rhodamine-HDL was then incubated with LRA in the dark for 30 minutes. After eluting proteins from the LRA, fluorescence from eluted rhodamine-PL was measured (excitation: 557 nm, emission: 571 nm). All data is expressed as a percentage of rhodamine-phospholipid fluorescence relative to the control containing HDL with no LRA (HDL, -LRA).

Supplement Table 1: HDL associated proteins by the method identified

Protein Identified	LRA	LRA+AALS	OSD
Afamin		x	x
Alpha-1-acid glycoprotein 1			x
Alpha-1-acid glycoprotein 2			x
Alpha-1-antichymotrypsin	x	x	x
Alpha-1-antitrypsin			x
Alpha-1B-glycoprotein		x	x
Alpha-2-antiplasmin	x	x	x
Alpha-2-HS-glycoprotein	x	x	x
Alpha-2-macroglobulin	x	x	x
Angiotensinogen	x	x	x
Antithrombin-III	x	x	x
Apolipoprotein A-I	x	x	x
Apolipoprotein A-II	x	x	x
Apolipoprotein A-IV	x	x	x
Apolipoprotein B-100		x	x
Apolipoprotein C-I	x	x	x
Apolipoprotein C-II	x	x	x
Apolipoprotein C-III	x	x	x
Apolipoprotein D			x
Apolipoprotein E	x	x	x
Apolipoprotein F			x
Apolipoprotein L1	x	x	x
Apolipoprotein M		x	x
C4b-binding protein alpha chain			x
Ceruloplasmin	x	x	x
Clusterin	x	x	x
Complement C1s subcomponent	x	x	x
Complement C2	x	x	x
Complement C3	x	x	x
Complement component C9	x	x	x
Complement factor B	x	x	x
Complement factor H	x	x	x
Fibrinogen alpha chain	x	x	x
Fibrinogen beta chain	x	x	x
Fibrinogen gamma chain	x	x	x
Fibronectin	x	x	x
Gelsolin	x	x	x
Haptoglobin	x	x	x
Haptoglobin-related protein	x	x	x
Hemoglobin subunit beta	x	x	x
Hemopexin	x	x	x
Heparin cofactor 2	x	x	x
Histidine-rich glycoprotein	x	x	x
Ig alpha-1 chain C region	x	x	x

Ig gamma-1 chain C region	x	x	x
Ig kappa chain C region	x	x	x
Ig lambda-2 chain C regions	x	x	x
Inter-alpha-trypsin inhibitor heavy chain H1	x	x	x
Inter-alpha-trypsin inhibitor heavy chain H2	x	x	x
Inter-alpha-trypsin inhibitor heavy chain H4	x	x	x
Kallistatin	x	x	x
Kininogen-1	x	x	x
Lumican		x	x
N-acetylmuramoyl-L-alanine amidase	x	x	x
Phosphatidylinositol-glycan-specific phospholipase D	x	x	x
Pigment epithelium-derived factor	x	x	x
Plasma kallikrein 1	x	x	x
Plasma protease C1 inhibitor	x	x	x
Plasminogen	x	x	x
Protein AMBP	x	x	x
Prothrombin	x	x	x
Retinol-binding protein 4		x	x
Serotransferrin		x	x
Serum albumin	x	x	x
Serum amyloid P-component			x
Serum paraoxonase/arylesterase 1	x	x	x
Transthyretin	x	x	x
Vitamin D-binding protein		x	x
Vitronectin	x	x	x
Zinc-alpha-2-glycoprotein			x