Supplement

Title: The Effects of Apolipoprotein B Depletion on HDL Subspecies Composition and Function

Running Title: ApoB Depletion

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Supplemental Methods

Trypsin digestion and LC-MS/MS analysis of apolipoproteins

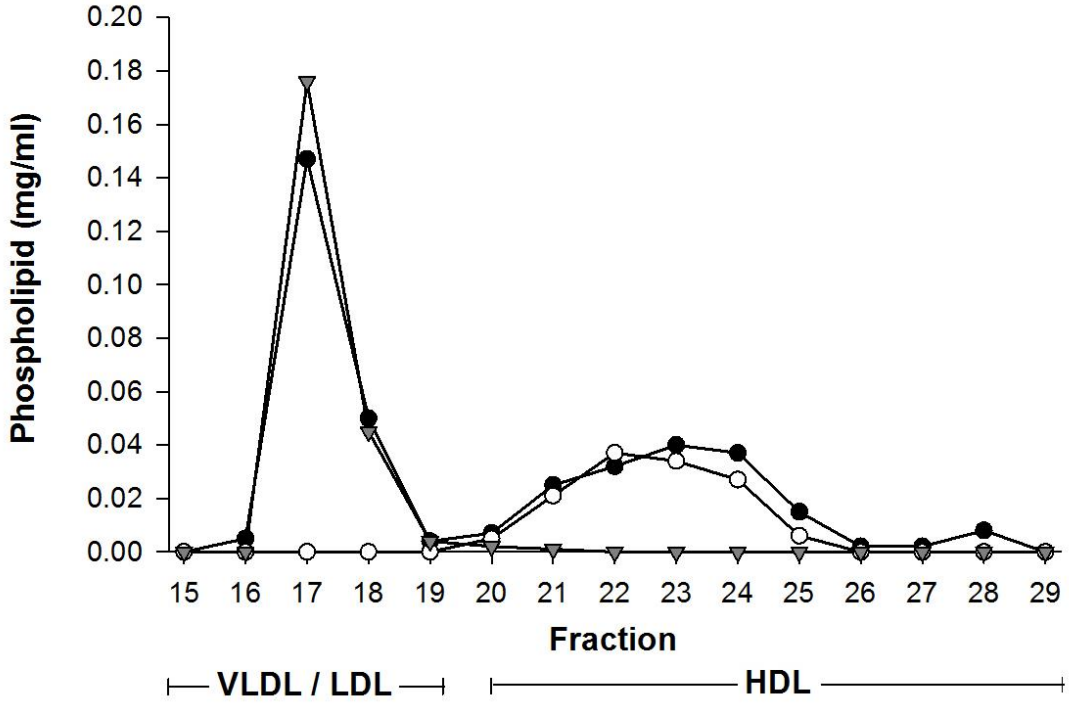
To a 100 µL aliquot of each of the 40 fractions, 50 µL Perfinity digest buffer containing 0.45% Zwittergent 3-12 (EMD Millipore, Billerica, MA, USA) was added and spiked with 5µL isotopically labeled target peptide mix (~10 pmol/mL). The trypsin digestion coupled LC separation was performed on an on-line column switching system, Perfinity (Shimadzu Scientific Instruments/Perfinity Biosciences Inc.), equipped with a Perfinity trypsin column (2.1 x 50 mm), a trapping column and an analytical column (HALO C18 core shell 5 x 2.1 mm 2.7 µm and 100 x 2.1 mm 2.7 µm particle size, respectively). From each diluted and spiked fraction 50 µL was injected on the on-line trypsin column at 25 µL/min flow rate collecting peptide cleavage products for 8 min on the trapping column. After digestion/trapping, the cleavage products were eluted to the analytical column and separated using an acetonitrile/0.1% formic acid in water solvent gradient program (3-95% at 0-7 min, and equilibration to 3% at 7-10 min) with 350 µL/min flow rate. The native peptide and the isotopically labeled peptide internal standard chromatograms were acquired in multiple reaction monitoring mode on a 6500 Qtrap instrument (AB Sciex, Foster City, CA, USA).

Supplementary Figures

Supplementary Figure 1: Overlay of phospholipid content from the triple Superdex Increase gel filtration chromatography separation of plasma (●) vs. ultracentrifugally-isolated LDL (▼) and HDL (○) from the same subject. Fractions 15-19 contain apoB and are comprised of LDL and VLDL. Fractions 20-29 are considered to be HDL-containing fractions because they are apoA-I rich and demonstrate particle diameters which are consistent with measurements for density-isolated HDL.

Supplementary Figure 2: Phospholipid and cholesterol distribution across lipoprotein subspecies of ultracentrifugally-isolated HDL before and after PEG apoB depletion. 1 ml density-isolated HDL (lacking LDL/VLDL) containing 9 mg/ml protein was apoB depleted using 400 µl 20% PEG (MW 6000). The resulting supernatant was collected and fractionated via triple Superdex Increase gel filtration chromatography. Comparison of volume-adjusted HDL control fractions (●) vs. PEG apoB depleted HDL fractions (○) in terms of a) phospholipid and b) cholesterol distribution.

Supplementary Figure I:



Supplementary Figure II: