SUPPLEMENTAL MATERIAL

Supplemental Methods

Multidimensional liquid chromatography-electrospray ionization (ESI) tandem mass spectrometric (MS/MS) analysis. Peptides from the tryptic digest of HDL samples (10 μg protein) were separated using 2-dimensional micro-liquid chromatography (LC) with strong cation exchange and reverse-phase capillary HPLC columns. The LC system was interfaced with a Finnigan LCQ Deca ProteomeX ion trap mass spectrometer (Thermo Electron Corporation) equipped with an orthogonal electrospray interface. A fully automated 10-step chromatography run with a quaternary Surveyor HPLC (Thermo Electron Corporation) was performed on each sample (15), using buffer A (0.1% v/v formic acid in water), buffer B (100% acetonitrile in 0.1% formic acid), buffer C (5% acetonitrile in 0.1% formic acid), and buffer D (ammonium chloride in buffer C). Peptides were eluted from the strong cation exchange column onto the reverse-phase column using increasing concentrations of ammonium chloride in buffer D (20, 30, 40, 50, 60, 70, 80, 120 and 600 mM). Peptides were eluted from the reverse-phase column at a flow rate of 2 μL/min using the following program: i) 95% buffer A and 5% buffer B for 5 min, ii) a linear gradient of 5% to 35% buffer B in buffer A for 90 min, iii) 35% buffer B and 65% buffer A for 15 min, iv) 20% buffer A and 80% buffer B for 10 min, v) a linear gradient of 80% to 5% buffer B in buffer A for 20 min, vi) 5% buffer B and 95% buffer A for 20 min. A survey scan from m/z 300 to m/z 1500 was initially performed, followed by data-dependent MS/MS analysis of the 3 most abundant ions.

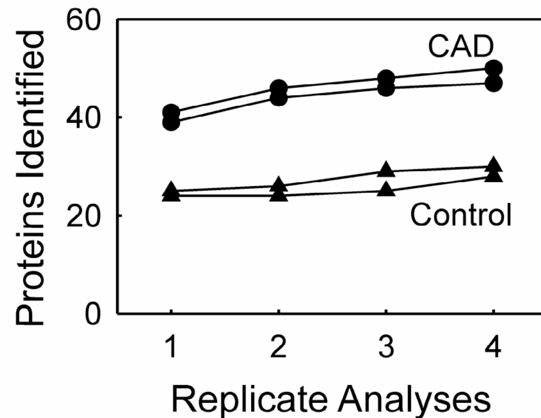
Tryptic digests of total HDL and apoA-I particles were analyzed by capillary LC (Paradigm MS4B, Michrom Bioresources Inc. Auburn, CA) coupled to a electrospray-

linear ion trap mass spectrometer (LTQ, Thermo Electron Corp., Waltham, MA). Tryptic digest (2 μg HDL protein) was injected onto a trap column (Paradigm Platinum Peptide Nanotrap, Michrom Bioresources Inc.), desalted for 5 min with 5% acetonitrile, 0.1% formic acid (50 μL/min), and then eluted onto an analytical reverse-phase column (0.150 x 150 mm, Magic C18AQ, 5 μm beads; Michrom Bioresources Inc.) and separated at a flow rate of 1 μL/min over 180 min using a linear gradient of 5 to 37% buffer E (5% acetonitrile, 0.1% formic acid) in buffer F (90% acetonitrile, 0.1% formic acid). Tandem mass spectra were acquired using date-dependent acquisition with one MS survey scan followed by MS/MS scans of the 8 most abundant peaks in the survey scan.

Shotgun proteomics of HDL identifies proteins reproducibly. Shotgun proteomics must often be repeated many times to correctly identify all the proteins in a complex mixture (1). For studies of material derived from patients, biological variability is an additional problem. Because LC-ESI-MS/MS is a time- and instrument-intensive procedure, we determined the number of analyses needed to reasonably assess protein composition.

Using ultracentrifugation, we isolated HDL from the blood of 2 healthy men and 2 men with established CAD. Each sample was digested with trypsin and then subjected to LC-ESI-MS/MS with data-dependent analysis. Our initial analysis identified ~24 proteins in the HDL from the 2 controls and ~40 proteins in the HDL from the 2 patients (Supplemental material; Fig. 1). Three additional analyses of the same samples increased the total number of proteins identified in each group by ~20%. These observations indicate that a single analysis of HDL gives a reasonable estimate of the number of

proteins that LC-ESI-MS/MS can identify and that the analyses yield reproducible results.



Supplemental Figure 1. Effect of repeated LC-ESI-MS/MS analysis on the number of proteins identified in plasma HDL. Total HDL was isolated by density gradient ultracentrifugation from the plasma of 2 control subjects and 2 subjects with established CAD. HDL proteins were precipitated with trichloroacetic acid, digested with trypsin, and desalted. Then each digest was subjected to 4 independent 2-dimensional LC-ESI-MS/MS analyses with an ion trap instrument.

Reference

1. Washburn, M.P., Ulaszek, R.R., and Yates, J.R., 3rd. 2003. Reproducibility of quantitative proteomic analyses of complex biological mixtures by multidimensional protein identification technology. *Anal Chem* 75:5054-5061.

Supplemental Table 1. HDL-associated proteins enriched in patients with CAD.

Protein	Peptide index	*P* Value
ApoC-IV	0.86	0.006
Paraoxonase 1	0.73	0.004
C3	0.65	0.03
ApoA-IV	0.58	0.002
ApoE	0.54	0.0003

Relative protein abundance was assessed initially using a peptide index of > 0.4 followed by statistical analysis. The *P* value was assessed by Student's t-test (peptide number) or Fisher's exact test (subject number).

Supplemental Table 2. Proteins detected by LC-ESI-MS/MS in HDL isolated from human atherosclerotic tissue and plasma of CAD patients.

Protein Description	Unique Peptides
Paraoxonase 1	26
C3	45
ApoE	118

Human atherosclerotic tissue was obtained at surgery from 6 subjects undergoing carotid endarterectomy. HDL was isolated from tissue by sequential density gradient ultracentrifugation. Proteins associated with 3 different pooled preparations of lesion HDL (prepared from 2 different individuals) were analyzed in duplicate by LC-ESI-MS/MS. Proteins were identified as described in the legend to Table 2 of the paper.