

Biochemical and Functional Characterization of Charge-defined Subfractions of High-density Lipoprotein From Normal Adults

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Contents of Supporting Information:

Table S-1: NanoLC-MS/MS analysis of the iTRAQ-labeled peptides detected in HDL subfractions

Table S-2: Molecular functions of the iTRAQ-labeled proteins identified by nanoLC-MS/MS

Experimental Section: Protein Digestion and iTRAQ Labeling

NanoLC-MS/MS

Protein Identification and Quantification

Table S-1. NanoLC-MS/MS analysis of the iTRAQ-labeled peptides detected in HDL subfractions

Accession	Protein	MW (kDa)	pI	Scores	SC (%)	H1/H3	H2/H3	H4/H3	H5/H3	H4/H1	H5/H1
A1AG2_HUMAN	Alpha-1-acid glycoprotein 2	23.6	23.7	31.1	4.5	0.71	0.84	2.02	2.5	2.82	3.51
A1AT_HUMAN	Alpha-1-antitrypsin	46.7	5.3	2095.1	42.3	0.61	1.49	1	1.39	1.65	2.29
A2AP_HUMAN	Alpha-2-antiplasmin	54.5	5.9	160.8	5.3	0.40	0.82	1.1	1.18	2.75	2.97
ABCD3_HUMAN	ATP-binding cassette	75.4	9.9	30.4	1.4	1.31	0.69	0.9	NA	0.69	NA
ACACB_HUMAN	Acetyl-CoA carboxylase 2	276.4	6.0	34.6	0.3	0.61	1.03	1.06	NA	1.75	NA
ADCY9_HUMAN	Adenylate cyclase type 9	150.6	7.3	69.7	0.7	0.64	1.30	0.95	NA	1.49	NA
ALBU_HUMAN	Serum albumin	69.3	5.9	4282.5	67.0	0.88	1.30	0.38	2.08	0.45	2.35
ANGT_HUMAN	Angiotensinogen	53.1	5.9	127.2	4.9	0.36	0.95	1.27	1.31	3.54	3.65
ANT3_HUMAN	Antithrombin-III	52.6	6.3	78.0	3.9	0.84	0.98	1.65	NA	1.96	NA
APBA2_HUMAN	Amyloid beta A4 precursor protein-binding family A member 2	82.5	4.6	35.5	2.4	1.28	1.15	0.4	NA	0.31	NA
APC7_HUMAN	Anaphase-promoting complex subunit 7	66.8	5.3	30.6	3.0	0.78	1.56	0.75	NA	0.97	NA
APOA1_HUMAN	Apolipoprotein A-I	30.8	5.5	8833.5	87.6	0.85	0.90	1.09	1.36	1.28	1.60
APOA2_HUMAN	Apolipoprotein A-II	11.2	7.1	1101.6	69.0	0.43	1.47	0.29	0.52	0.67	1.20
APOA_HUMAN	Apolipoprotein(a)	501.0	5.5	606.2	4	NA	NA	NA	3.19	NA	NA
APOB_HUMAN	Apolipoprotein B-100	515.3	6.6	4012.2	20.4	0.92	0.65	0.71	0.95	0.75	1.03
APOC1_HUMAN	Apolipoprotein C-I	9.3	9.3	561.6	37.3	3.50	0.98	0.93	1.23	0.27	0.35
APOC2_HUMAN	Apolipoprotein C-II	11.3	4.5	419.6	54.5	0.35	2.33	0.51	0.85	2.01	2.40
APOC3_HUMAN	Apolipoprotein C-III	10.8	5.1	1055.0	58.6	0.25	1.12	0.23	0.69	0.96	2.81
APOC4_HUMAN	Apolipoprotein C-IV	14.5	10.4	151.1	23.6	2.44	0.95	0.9	1.21	0.37	0.50
APOD_HUMAN	Apolipoprotein D	21.3	4.9	775.2	39.2	0.44	1.67	0.34	0.59	0.81	1.34
APOE_HUMAN	Apolipoprotein E	36.1	5.5	1803.2	60.9	0.57	0.56	1.38	1.51	2.41	2.63
APOF_HUMAN	Apolipoprotein F	35.4	5.3	123.6	7.7	0.47	1.08	0.99	3.86	2.10	8.18
APOL1_HUMAN	Apolipoprotein L1	43.9	5.5	407.2	18.1	1.28	0.87	0.78	0.8	0.61	0.63
APOM_HUMAN	Apolipoprotein M	21.2	5.6	764.9	54.8	0.37	0.71	1.12	1.26	3.32	3.37

BCAR1_HUMAN	Breast cancer anti-estrogen resistance protein 1	93.3	5.3	33.2	0.8	0.76	0.69	0.87	0.8	1.13	1.05
CB047_HUMAN	Uncharacterized protein C2orf47, mitochondrial	32.5	10.1	32.6	2.7	0.58	0.94	1.24	2.13	2.15	3.70
CENPF_HUMAN	Centromere protein F	367.5	4.9	30.8	0.3	1.15	0.70	1.14	NA	0.99	NA
CF163_HUMAN	Uncharacterized protein C6orf163	38.5	6.5	33.6	2.4	1.08	0.47	0.96	NA	0.88	NA
CHM4B_HUMAN	Charged multivesicular body protein 4b	24.9	4.6	35.0	3.1	0.81	1.16	0.87	0.94	1.07	1.16
CI050_HUMAN	Uncharacterized protein C9orf50	47.6	11.6	34.8	2.1	1.09	1.09	0.98	1.18	0.91	1.09
CLUS_HUMAN	Clusterin	52.5	5.9	514.7	23.8	NA	0.83	1.54	2.82	3.51	NA
CNTRL_HUMAN	Centriolin	268.7	5.3	102.5	0.8	0.94	0.97	1.04	NA	1.10	NA
CO3_HUMAN	Complement C3	187.0	6.0	517.1	9.4	0.57	0.76	0.95	1.19	1.53	2.10
CO4A_HUMAN	Complement C4-A	192.7	6.7	378.6	5.5	NA	NA	NA	2.66	NA	NA
CP4V2_HUMAN	Cytochrome P450 4V2	60.7	7.8	39.3	1.1	0.49	0.99	0.39	NA	0.80	NA
DESP_HUMAN	Desmoplakin	331.6	6.4	30.4	0.2	0.79	0.83	1.03	NA	1.30	NA
EFCB6_HUMAN	EF-hand calcium-binding domain-containing protein 6	172.8	9.5	33.3	0.5	1.45	0.61	2.07	NA	1.42	NA
FAAH2_HUMAN	Fatty-acid amide hydrolase 2	58.3	9.9	38.5	2.6	2.98	1.16	0.76	NA	0.26	NA
FIBG_HUMAN	Fibrinogen gamma chain	51.5	5.3	45.2	3.3	1.04	0.84	0.99	NA	0.96	NA
FIG4_HUMAN	Polyphosphoinositide phosphatase	103.6	6.5	30.3	1.1	0.46	0.85	0.88	0.83	1.92	1.80
GRAM4_HUMAN	GRAM domain-containing protein 4	66.4	9.6	30.5	1.2	0.84	0.53	0.27	NA	0.32	NA
GXLT1_HUMAN	Glucoside xylosyltransferase 1	50.5	9.7	32.0	3.0	0.39	2.38	0.44	NA	1.13	NA
HERC6_HUMAN	Probable E3 ubiquitin-protein ligase HERC6	115.1	9.2	34.0	0.7	0.72	0.95	0.67	NA	0.93	NA
HPT_HUMAN	Haptoglobin	45.2	6.1	394.5	19.0	0.55	0.68	1.46	1.47	2.66	2.68
HPTR_HUMAN	Haptoglobin-related protein	39.0	6.7	543.1	34.8	0.41	0.63	1.72	1.58	3.97	3.84
IGHA1_HUMAN	Ig alpha-1 chain C region	37.6	6.1	220.1	13.0	0.62	0.78	0.97	1.31	1.56	2.11
IGHG1_HUMAN	Ig gamma-1 chain C region	36.1	9.4	183.5	15.2	1.10	1.03	1.37	1.17	1.26	1.07
IGHG2_HUMAN	Ig gamma-2 chain C region	35.9	8.8	151.7	11.7	1.23	1.35	1.34	1.32	1.08	1.07
IGHM_HUMAN	Ig mu chain C region	49.3	6.4	144.8	2	NA	NA	NA	5.92	NA	NA

IGKC_HUMAN	Ig kappa chain C region	11.6	5.5	267.7	32.1	1.54	0.83	0.8	1.69	0.51	1.10
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	103.3	6.5	86.5	2.5	0.51	0.73	0.58	0.67	1.14	1.32
K1C10_HUMAN	Keratin, type I cytoskeletal 10	58.8	5.0	220.0	11.6	0.66	1.04	1.79	2.31	3.34	3.53
K1C17_HUMAN	Keratin, type I cytoskeletal 17 2	48.1	4.8	63.6	4.6	0.87	1.28	1.79	2.11	2.06	2.44
K1C9_HUMAN	Keratin, type I cytoskeletal 9	62.0	5.0	120.2	7.9	0.68	0.97	1.41	1.73	2.08	2.55
K22E_HUMAN	Keratin, type II cytoskeletal 2 epidermal	65.4	8.9	244.8	11.6	0.64	0.99	1.7	2.35	2.67	3.68
K2C1_HUMAN	Keratin, type II cytoskeletal 1	66.0	8.8	550.8	17.1	0.56	0.91	1.49	2.21	2.67	3.96
LAC3_HUMAN	Ig lambda-3 chain C regions	11.2	7.7	92.0	28.3	0.71	0.95	0.79	2.8	1.11	3.94
LCAT_HUMAN	Phosphatidylcholine-sterol acyltransferase	49.5	5.7	245.3	13.4	0.28	0.41	2.45	2.17	8.76	7.73
LRIQ1_HUMAN	Leucine-rich repeat and IQ domain-containing protein 1	199.2	5.8	34.2	0.8	0.56	1.67	0.29	NA	0.52	NA
LYAM1_HUMAN	L-selectin OS	42.2	6.2	40.1	2.2	0.43	0.51	1.51	1.46	3.55	3.42
MAT1_HUMAN	CDK-activating kinase assembly factor MAT1	35.8	5.7	34.8	2.6	0.59	0.85	0.71	NA	1.21	NA
NALP2_HUMAN	NACHT, LRR and PYD domains-containing protein 2	120.4	5.7	34.3	0.7	0.92	0.94	1.28	1.08	1.38	1.17
NEB1_HUMAN	Neurabin-1	123.3	4.9	31.3	0.6	0.73	1.09	0.89	NA	1.21	NA
Lp-PLA2_HUMAN	Platelet-activating factor acetylhydrolase	50.0	7.9	38.8	1.8	0.84	0.88	1.29	NA	1.54	NA
PCYOX_HUMAN	Prenylcysteine oxidase 1	56.6	5.8	350.0	14.5	0.51	0.68	0.8	0.71	1.57	1.40
PEDF_HUMAN	Pigment epithelium-derived factor	46.3	6.0	48.9	3.3	0.61	1.75	0.26	NA	0.43	NA
PGPS1_HUMAN	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase, mitochondrial	62.7	9.8	30.5	2.0	0.63	1.20	0.77	NA	1.21	NA
PLTP_HUMAN	Phospholipid transfer protein	54.7	6.6	125.9	8.7	0.57	0.54	1.12	1.45	1.97	2.56
PLXA3_HUMAN	Plexin-A3	207.6	7.2	37.5	0.4	1.27	0.47	1.26	NA	1.00	NA

PON1_HUMAN	Serum paraoxonase/arylesterase 1	39.7	5.0	291.6	16.9	0.41	0.57	2.06	4.09	5.00	9.89
PON3_HUMAN	Serum paraoxonase/lactonase 3	39.6	5.1	243.5	15.3	NA	NA	NA	3.56	NA	NA
PRKRA_HUMAN	Interferon-inducible double stranded RNA-dependent protein kinase activator A	34.4	9.6	37.1	4.8	0.68	0.99	1.05	NA	1.55	NA
REV1_HUMAN	DNA repair protein REV1	138.2	9.5	36.6	0.8	1.09	0.82	1.07	NA	0.98	NA
RPA34_HUMAN	DNA-directed RNA polymerase I subunit RPA34	55.0	9.4	34.5	2.4	0.71	0.57	0.58	NA	0.82	NA
RUFY2_HUMAN	RUN and FYVE domain-containing protein 2	75.0	6.1	69.4	1.1	0.97	1.14	1.14	1.1	1.17	1.13
SAA_HUMAN	Serum amyloid A protein	13.5	6.4	242.2	29.5	0.91	1.12	0.7	0.81	0.76	0.89
SAA4_HUMAN	Serum amyloid A-4 protein	14.7	9.8	639.7	30.0	2.09	0.96	0.68	0.75	0.33	0.36
SIA8C_HUMAN	Sia-alpha-2,3-Gal-beta-1,4-GlcNAc-R:alpha 2,8-sialyltransferase	43.9	10.1	34.3	2.9	1.18	0.60	1.13	NA	0.96	NA
SYNE1_HUMAN	Nesprin-1	1010.4	5.3	77.8	0.1	1.02	0.76	0.97	1.52	0.95	1.48
TRNK1_HUMAN	TPR and ankyrin repeat-containing protein 1	336.0	6.3	31.3	0.3	1.16	1.45	1.29	1.19	1.11	1.03
TRY1_HUMAN	Trypsin-1	26.5	6.1	140.1	4.0	1.14	1.19	0.9	1.13	0.78	0.99
TTHY_HUMAN	Transthyretin	15.9	5.4	308.6	52.4	0.63	0.60	2.27	2.36	3.51	3.74
TXLNB_HUMAN	Beta-taxilin	76.5	4.8	33.2	1.5	NA	NA	NA	1.92	NA	NA
UT14C_HUMAN	U3 small nucleolar RNA-associated protein 14 homolog C	87.1	6.7	30.2	1.4	NA	NA		1.48		NA
VTNC_HUMAN	Vitronectin	54.3	5.5	258.8	15.1	NA	NA	NA	3.18	NA	NA
YK041_HUMAN	Putative uncharacterized protein ENSP00000334305	84.9	8.8	30.4	1.3	1.12	0.81	1.02	NA	0.91	NA
ZEP1_HUMAN	Zinc finger protein 40	296.7	8.9	32.8	0.4	0.79	1.14	0.78	NA	0.99	NA
ZN175_HUMAN	Zinc finger protein 175	81.6	10.1	30.4	1.1	NA	NA	NA	4.84	NA	NA

MW, molecular weight; pI, isoelectric point; SC, sequence coverage.

Table S-2. Molecular functions of the iTRAQ-labeled proteins identified by nanoLC-MS/MS

Function	Gene	Protein	H1/H3	H2/H3	H4/H3	H5/H3
Lipid metabolism						
	<i>apoAI</i>	Apolipoprotein A-I	0.85	0.90	1.09	1.36
	<i>apoAII</i>	Apolipoprotein A-II	0.43	1.47	0.29	0.52
	<i>apoB</i>	Apolipoprotein B-100	0.92	0.65	0.71	0.95
	<i>apoC1</i>	Apolipoprotein C-I	3.50	0.98	0.93	1.23
	<i>apoC2</i>	Apolipoprotein C-II	0.35	2.33	0.51	0.85
	<i>apoC3</i>	Apolipoprotein C-III	0.25	1.12	0.23	0.69
	<i>apoC4</i>	Apolipoprotein C-IV	2.44	0.95	0.9	1.21
	<i>apoD</i>	Apolipoprotein D	0.44	1.67	0.34	0.59
	<i>apoE</i>	Apolipoprotein E	0.57	0.56	1.38	1.51
	<i>apoF</i>	Apolipoprotein F	0.47	1.08	0.99	3.86
	<i>CLU\apoJ</i>	Clusterin\Apolipoprotein J	NA	0.83	1.54	2.82
	<i>apoL1</i>	Apolipoprotein L1	1.28	0.87	0.78	0.8
	<i>apoM</i>	Apolipoprotein M	0.37	0.71	1.12	1.26
	<i>PLTP</i>	Phospholipid transfer protein	0.57	0.54	1.12	1.45
	<i>LCAT</i>	Phosphatidylcholine-sterol acyltransferase	0.28	0.41	2.45	2.17
	<i>Lp-PLA2</i>	Lipoprotein-associated phospholipase A2	0.84	0.88	1.29	NA
	<i>PON1</i>	Serum paraoxonase 1	0.41	0.57	2.06	4.09
	<i>PON3</i>	Serum paraoxonase 3	NA	NA	NA	3.56
	<i>LPA</i>	Apolipoprotein(a)	NA	NA	NA	3.19
Antioxidant						
	<i>apoAI</i>	Apolipoprotein A-I	0.85	0.90	1.09	1.36
	<i>apoM</i>	Apolipoprotein M	0.37	0.71	1.12	1.26
	<i>LCAT</i>	Phosphatidylcholine-sterol acyltransferase	0.28	0.41	2.45	2.17
	<i>Lp-PLA2</i>	Lipoprotein-associated phospholipase A2	0.84	0.88	1.29	NA
	<i>PON1</i>	Serum paraoxonase 1	0.41	0.57	2.06	4.09
	<i>PON3</i>	Serum paraoxonase 3	NA	NA	NA	3.56
Complement						
	<i>CLU\apoJ</i>	Clusterin\Apolipoprotein J	NA	0.83	1.54	2.82
	<i>C3</i>	Complement C3	0.57	0.76	0.95	1.19
	<i>C4A</i>	Complement C4-A	NA	NA	NA	2.66
	<i>VTN</i>	Vitronectin	NA	NA	NA	3.18
Acute-phase response						
	<i>SAA4</i>	Serum amyloid A-4 protein	2.09	0.96	0.68	0.75
	<i>SAA1</i>	Serum amyloid A protein	0.91	1.12	0.7	0.81
	<i>ITIH4</i>	Inter-alpha-trypsin inhibitor heavy chain H4	0.51	0.73	0.58	0.67
	<i>SERPINA1</i>	Alpha-1-antitrypsin	0.61	1.49	1	1.39
	<i>SERPINF2</i>	Alpha-2-antiplasmin	0.40	0.82	1.1	1.18

	<i>SERPINC1</i>	Antithrombin-III	0.84	0.98	1.65	NA
	<i>ORM2</i>	Alpha-1-acid glycoprotein 2	0.71	0.84	2.02	2.5
	<i>HP</i>	Haptoglobin	0.55	0.68	1.46	1.47
	<i>HPR</i>	Haptoglobin-related protein	0.41	0.63	1.72	1.58
	<i>TTR</i>	Transthyretin	0.63	0.60	2.27	2.36
Protease inhibitor						
	<i>ITIH4</i>	Inter-alpha-trypsin inhibitor heavy chain H4	0.51	0.73	0.58	0.67
	<i>SERPINA1</i>	Alpha-1-antitrypsin	0.61	1.49	1	1.39
	<i>SERPINF2</i>	Alpha-2-antiplasmin	0.40	0.82	1.1	1.18
	<i>SERPINC1</i>	Antithrombin-III	0.84	0.98	1.65	NA
	<i>AGT</i>	Angiotensinogen	0.36	0.95	1.27	1.31
Immune response						
	<i>IGHA1</i>	Ig alpha-1 chain C region	0.62	0.78	0.97	1.31
	<i>IGHG1</i>	Ig gamma-1 chain C region	1.10	1.03	1.37	1.17
	<i>IGHG2</i>	Ig gamma-2 chain C region	1.23	1.35	1.34	1.32
	<i>IGKC</i>	Ig kappa chain C region	1.54	0.83	0.8	1.69

Colors indicate the relative abundance of each protein: green (low), yellow (medium), and red (high). NA,

not available.

EXPERIMENTAL SECTION

Protein Digestion and iTRAQ Labeling. HDL subfractions H1 through H5 were individually dissolved in 25 mM triethylammonium bicarbonate and heated to 90°C for 20 min. The denatured proteins were reduced and alkylated by using solutions provided in the iTRAQ experiment kit. Trypsin was added to the protein solution at an enzyme-to-substrate ratio of 1:50 (wt/wt), and the mixture was incubated for 12 hours at 37°C. Digested HDL samples were then labeled with iTRAQ reagents according to the manufacturer's protocol. The first comparison experiment consisted of subfractions H1 through H4, which were labeled with reagents 114 through 117, respectively. The second experiment consisted of subfractions H3 and H5, which were labeled with reagents 114 and 117, respectively. For each experiment, 20 μ L of label reagent was applied to 30 μ g of subfraction digest. Samples in each comparison experiment were mixed and dried by using a centrifugal concentrator (miVac Duo Concentrator; Genevac, Stone Ridge, NY). Before nanoflow liquid chromatography tandem mass spectrometry (nanoLC-MS/MS) was performed, the samples were desalted with C18 SPE cartridges (Waters, Milford, MA).

NanoLC-MS/MS. NanoLC-MS/MS was performed by using a nanoflow, ultra-high performance liquid chromatography system (UltiMate 3000 RSLCnano system, Dionex, Amsterdam, Netherlands) coupled with a hybrid Q-TOF mass spectrometer (maXis impact, Bruker Daltonics, Billerica, MA). Samples were injected into a home-made tunnel-frit trap column (C18, 5 μ m, 180 μ m x 20 mm) with a flow rate of 10 μ L/min for a duration of 4 min.¹ The trapped analytes were separated by using a commercial analytical column (Acclaim PepMap C18, 2 μ m, 100 Å, 75 μ m x 250 mm, Thermo Scientific) with a flow rate of 300 nL/min. An acetonitrile/water gradient of 1% to 35% within 76 min was used for peptide separation. For MS

detection, peptides that had a charge of 2+, 3+, or 4+ and an intensity greater than 50 counts were selected for data-dependent acquisition, which was set to 1 full MS scan (400-1600 m/z) with 1 Hz and was switched to 10 product ion scans (100-1990 m/z) with 5 Hz.

Protein Identification and Quantification. NanoLC-MS/MS spectra were deisotoped, centroided, and converted to .xml files by using DataAnalysis software (version 4.1, Bruker Daltonics). To identify proteins, the mass spectra obtained were compared to those in the SwissPort database (release 51.0) by using the MASCOT search algorithm (version 2.2.07). The search parameters for MASCOT included peptide and MS/MS mass tolerance set to 0.05 Da, taxonomy–human, enzyme–trypsin, fixed modification–methylthio (Cys), variable modification–oxidation (Met), deamidation (Asn/Gln), and iTRAQ4plex (Lys/Tyr/N-term). Peptides were identified if their MASCOT individual ion score was higher than 30. The protein ratio with iTRAQ labeling was processed and calculated by using ProteinScape (Bruker Daltonics). Gene ontology analysis (<http://david.abcc.ncifcrf.gov/>)²⁻³ was used to classify the detected proteins on the basis of molecular function.

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