Supplemental Material

Niacin increases atherogenic proteins in HDL of statin-treated subjects

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Supplemental Methods

Discovery study: targeted proteomic analyses of CPC cohort. Digested HDL (250 ng protein) was quantified by parallel reaction monitoring, an approach that depends only on the relative abundance and detection efficiency of peptides in MS/MS. A nanoACQUITY UPLC (Waters, Milford, MA) was used for the separation, with a linear gradient of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Samples were desalted on an in-house packed C18 trap column (Waters XBridge BEH C18, 5 µm, 0.075 x 40 mm) for 6.5 minutes at 3 µL/min in 99% solvent A, and then separated using a C18 column packed in-house (Waters XBridge BEH C18, 3.5 µm, 0.075 x 120 mm) with an integrated electrospray emitter pulled by a laser micropipette puller (Sutter Instrument, Novato, CA). Peptides were eluted from the trap column onto the analytical column at a flow rate of 0.6 µL/min and separated using multi-step gradient as follows: 1% to 7% solvent B in 2 minutes; 7% to 25% solvent B in 17 minutes; 25% to 35% solvent B in 3 minutes. The column was subsequently washed for 3 minutes at 80% B and re-equilibrated at 99% A for 11 minutes. The column was kept at 50ºC. Experiments were performed using a Q Exactive mass spectrometer (Thermo Scientific, San Jose, California). The resolution was set at 17,500 (at m/z 200), the active gain control target at $5x10^4$, the maximum fill time at 50 ms, and the individual isolation window at 2 Th. Normalized collision energy of 25 was employed for fragmentation. A scheduled (3-minute window) inclusion list containing *m/z* of precursor peptides of interest and corresponding retention times was generated using Skyline software [21].

Validation study: targeted proteomic analyses of AIM-HIGH subjects - Tryptic digests of HDL were desalted on a C18 trapping column (Xbridge BEH C18 100 Å, 5 µm, 0.1 x 40 mm, Waters) (trapping flow rate 4 µL/min), separated on a capillary analytical column (XBridge BEH C18 100 Å, 3.5 µm, 120x0.075 mm, Waters) with a 30 min linear gradient of acetonitrile, 0.1% formic acid (7-35%) in 0.1% formic acid in water at a flow rate of 0.6 µL/min using a nanoAquity UPLC (Waters, MA) and analyzed in a Thermo TSQ Vantage triple-quadrupole mass spectrometer with electrospray ionization. The instrument was operated in selected reaction monitoring mode with 10 ms dwell time and the peptides were monitored with collision energies optimized to maximize the signal. Peak areas were integrated using Skyline software [21].

Selection of HDL peptides for targeted quantification. Thirty-one proteins consistently detected in HDL in previous studies were selected for targeted quantification [8, 9, 15, 22, 23]. Based on these studies and considering the observed frequency, two to five peptides per protein were selected for parallel reaction monitoring quantification. We excluded peptides that are susceptible to *ex vivo* modification (e.g., containing methionine) and peptides with missing cleavage sites. Supplemental Table I shows a list of proteins and peptides monitored for targeted quantification by parallel reaction monitoring quantification PRM.

Next, we assessed the relationship among levels of individual peptides of each proteins using Pearson correlation. For each protein, two peptides with the best correlation were selected. Supplemental Table II shows the correlations between peptides for each protein. The chosen surrogate peptide is showed in the first column.

We used the same peptides chosen for the CPC study to serve as surrogate for proteins quantified in AIM-HIGH. Alpha-1-Microglobulin/Bikunin Precursor (AMBP) and Platelet basic protein (PPBP) were not quantified in the AIM-HIGH study. Moreover, the surrogate peptides used for the CPC study were not measured in AIM-HIGH samples for apolipoprotein E (APOE), apolipoprotein L1 (APOL1) and Phosphatidylcholine-sterol acyltransferase (LCAT). Therefore, different peptides were selected for these proteins (Supplemental Table II).

Among the proteins selected for HDL quantification, two different protein families containing highly homologous sequences were targeted. Serum amyloid A (SAA) 1, 2 and 4 were quantified for the SAA family, however, only SAA 1 and 2 share significantly homology. Two peptides shared by SAA1 and SAA2 were quantified, and they are termed as SAA1/2 peptides (Supplemental Table II). For the peptidase S1 family, one peptides shared by the proteins haptoglobin (HP) and haptoglobin-related protein (HPR) was quantified. This joint quantification is termed as HP/HPR in Supplemental Table II. In addition, one unique peptide to the protein HP and one to the protein HPR were also integrated (represented respectively as HP and HPR in Supplemental Table II).

Supplemental Tables

Supplemental Table I. List of proteins and peptides monitored by parallel reaction monitoring in CPC study.

Protein Name	Peptide Sequence
AMBP	GECVPGEQEPEPILIPR
AMBP	ETLLQDFR
APOA1	DLATVYVDVLK
APOA1	DYVSQFEGSALGK
APOA1	VQPYLDDFQK
APOA2	EQLTPLIK
APOA2	SPELQAEAK
APOA2	EPCVESLVSQYFQTVTDYGK
APOA4	LGEVNTYAGDLQK
APOA4	SELTQQLNALFQDK
APOB	IEIPLPFGGK
APOB	SVSLPSLDPASAK
APOC1	EFGNTLEDK

APOC1	EWFSETFQK
APOC1	TPDVSSALDK
APOC2	TAAQNLYEK
APOC2	TYLPAVDEK
APOC2	ESLSSYWESAK
APOC3	DALSSVQESQVAQQAR
APOC3	LTPYADEFK
APOC3	DYWSTVK
APOC3	GWVTDGFSSLK
APOC4	AWFLESK
APOC4	ELLETVVNR
APOC4	DGWQWFWSPSTFR
APOC4	DLGPLTK
APOD	IPTTFENGR
APOD	NPNLPPETVDSLK
APOD	VLNQELR
APOE	AATVGSLAGQPLQER
APOE	SELEEQLTPVAEETR
APOE	LGPLVEQGR
APOF	SGVQQLIQYYQDQK
APOF	SLPTEDCENEK
APOF	SYDLDPGAGSLEI
APOL1	LNILNNNYK
APOL1	VTEPISAESGEQVER
APOL1	VAQELEEK
APOL1	ALDNLAR
APOM	AFLLTPR
APOM	DGLCVPR
APOM	SLTSCLDSK
C3	TGLQEVEVK
C3	TIYTPGSTVLYR
CETP	ASYPDITGEK
CETP	VIQTAFQR
CETP	GTSHEAGIVCR
CLU	ASSIIDELFQDR
CLU	LFDSDPITVTVPVEVSR
CLU	ELDESLQVAER
HBB	LLVVYPWTQR
HBB	SAVTALWGK
HP	VTSIQDWVQK
HP/HPR	GSFPWQAK
HP/HPR	LPECEAVCGKPK

HPR	VGYVSGWGQSDNFK
LCAT	SSGLVSNAPGVQIR
LCAT	STELCGLWQGR
LCAT	LEPGQQEEYYR
LCAT	TYSVEYLDSSK
LPA	GTLSTTITGR
LPA	TPAYYPNAGLIK
PCYOX1	LFLSYDYAVK
PCYOX1	LVCSGLLQASK
PLTP	AVEPQLQEEER
PLTP	FLEQELETITIPDLR
PON1	IFFYDSENPPASEVLR
PON1	IQNILTEEPK
PON1	STVELFK
PON1	SFNPNSPGK
PON3	AQALEISGGFDK
PON3	SVNDIVVLGPEQFYATR
PON3	LLNYNPEDPPGSEVLR
PPBP	NIQSLEVIGK
PPBP	ICLDPDAPR
RBP4	LLNLDGTCADSYSFVFSR
RBP4	YWGVASFLQK
SAA1/2	DPNHFRPAGLPEKY
SAA1/2	SFFSFLGEAFDGAR
SAA4	FRPDGLPK
SAA4	GPGGVWAAK
VTN	DVWGIEGPIDAAFTR
VTN	FEDGVLDPDYPR
VTN	GQYCYELDEK

Supplemental Table II. Pearson correlation between peptide pairs for HDL proteins quantified by parallel reaction monitoring in CPC subjects. The surrogate peptide is shown in the first column. The last column shows surrogate peptide for AIM-HIGH trial.

Protein Name	Surrogate Peptide	Second Peptide	r	AIM-HIGH
AMBP	ETLLQDFR	GECVPGEQEPEPILIPR	0.716	-
APOA1	VQPYLDDFQK	DYVSQFEGSALGK	0.873	VQPYLDDFQK
APOA2	SPELQAEAK	EQLTPLIK	0.801	SPELQAEAK
APOA4	LGEVNTYAGDLQK	SELTQQLNALFQDK	0.864	LGEVNTYAGDLQK
APOB	IEIPLPFGGK	SVSLPSLDPASAK	0.874	IEIPLPFGGK
APOC1	TPDVSSALDK	EFGNTLEDK	0.781	TPDVSSALDK
APOC2	TAAQNLYEK	TYLPAVDEK	0.941	TAAQNLYEK
APOC3	DALSSVQESQVAQQAR	GWVTDGFSSLK	0.869	DALSSVQESQVAQQAR
APOC4	AWFLESK	ELLETVVNR	0.901	AWFLESK
APOD	VLNQELR	NPNLPPETVDSLK	0.755	VLNQELR
APOE	LGPLVEQGR	AATVGSLAGQPLQER	0.919	AATVGSLAGQPLQER
APOF	SYDLDPGAGSLEI	SLPTEDCENEK	0.507	SYDLDPGAGSLEI
APOL1	ALDNLAR	VAQELEEK	0.947	VTEPISAESGEQVER
APOM	AFLLTPR	SLTSCLDSK	0.797	AFLLTPR
C3	TGLQEVEVK	TIYTPGSTVLYR	0.826	TGLQEVEVK
CETP	VIQTAFQR	ASYPDITGEK	0.949	VIQTAFQR
CLU	LFDSDPITVTVPVEVSR	ASSIIDELFQDR	0.921	LFDSDPITVTVPVEVSR
HBB	SAVTALWGK	LLVVYPWTQR	0.997	SAVTALWGK
HP	VTSIQDWVQK			VTSIQDWVQK
HP/HPR	GSFPWQAK	LPECEAVCGKPK	0.956	GSFPWQAK
HPR	VGYVSGWGQSDNFK			VGYVSGWGQSDNFK
LCAT	TYSVEYLDSSK	LEPGQQEEYYR	0.855	LEPGQQEEYYR
LPA	GTLSTTITGR	TPAYYPNAGLIK	0.979	GTLSTTITGR
PCYOX1	LVCSGLLQASK	LFLSYDYAVK	0.832	LVCSGLLQASK
PLTP	FLEQELETITIPDLR	AVEPQLQEEER	0.884	FLEQELETITIPDLR
PON1	IQNILTEEPK	STVELFK	0.913	IQNILTEEPK
PON3	LLNYNPEDPPGSEVLR	AQALEISGGFDK	0.833	LLNYNPEDPPGSEVLR
PPBP	ICLDPDAPR	NIQSLEVIGK	0.979	-
RBP4	YWGVASFLQK	LLNLDGTCADSYSFVFSR	0.821	YWGVASFLQK
SAA 1 / 2	SFFSFLGEAFDGAR	DPNHFRPAGLPEKY	0.975	SFFSFLGEAFDGAR
SAA4	GPGGVWAAK	FRPDGLPK	0.781	GPGGVWAAK
VTN	FEDGVLDPDYPR	GQYCYELDEK	0.893	FEDGVLDPDYPR

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
NA				

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	NA				
Parent - Female	NA				

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
NA					

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL
NA			

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
NA			

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
NA		

Other

Description	Source / Repository	Persistent ID / URL
NA		