SUPPLEMENTARY INFORMATION

Materials and Methods

Dried blood spot sample preparation, extraction and digestion

Dried blood spots of EDTA-WB were generated by spotting onto Whatman 903 filter paper. Each spot was formed using a single channel pipette where a 65 µL droplet of EDTA-WB formed on the pipette tip was placed on the filter paper without touching the pipet tip to the paper. The spots were dried for 3 h at 25°C, then placed in re-sealable plastic bags containing 300 g of silica desiccant (Electron Microscope Sciences) and stored at -80°C. Capillary dried blood spots were obtained from human subjects using incision lancets [Tenderfoot (ITC Med) or Quickheel (BD)] on the volunteer's finger of choice to produce a single drop of blood that was placed on Whatman 903 filter paper, taking great care to avoid touching the finger to the paper. The first drop of blood following incision was wiped away and the next drop was spotted. Five spots were obtained from each volunteer by a trained phlebotomist. The spots were then dried and stored in a similar fashion to the EDTA-WB DBS described above. Prior to processing, the DBS samples were removed from -80°C and allowed to warm to room temperature before being removed from their plastic bag. Spots were punched using a BSD700 semi-automated dried sample puncher (BSD Robotics, Brisbane, QLD, Australia). A 3.2 mm (1/8") diameter punch was punched from the DBS into a deep-well plate (Grenier Bio-One), which was then sealed using a silicon plate mat (Grenier Bio-One) and stored at -80°C.

Prior to extraction and digestion of blood spots, the microtiter plate containing the DBS was brought to room temperature. A DBS punch was transferred from the plate to a 1.5 mL Safe-Lock low protein binding microfuge tube (Eppendorf) using stainless steel forceps. Alternatively, 10 µL of plasma was added to a 1.5 mL Safe-Lock low protein binding microfuge tube. Then 70 µL (60 µL for plasma) of a 100 mM ammonium bicarbonate (AMBIC) buffer solution containing 14.3 ng/µL ^{15}N-labeled ApoA-I (IS_prot) was quantitatively transferred to the DBS containing tube. Then 70 µL of trifluoroethanol (TFE) (Sigma-Aldrich) was added to the tube, which was then vortexed for one hour on an Eppendorf thermomixer at 65°C at 1400 rpm. The extraction step was not performed on plasma samples. Reduction of proteins in solution was accomplished by adding 2 µL of 500 mM DTT to the AMBIC/TFE/IS_prot solution and vortexed at 65°C for 1 h at 1400 rpm. Alkylation of cysteine residues was then performed by allowing the samples to cool to RT followed by the addition of 8 µL of 500 mM iodoacetamide to the reduced protein solution and incubated for 30 min at RT in the dark. Residual iodoacetamide was quenched by the addition of 2 µL of 500 mM DTT. Prior to digestion, the TFE concentration was reduced to 5.5% by the addition of 1200 µL of 27.5 mM AMBIC solution containing 104 nM IS_pep to the reduced and alkylated sample. The IS_pep stock solution contained equimolar quantities of each of the seven SIS peptides. Then 20 µL of 2.0 mg/mL trypsin (Worthington) in 1mM HCL was added and vortexed at 37^{0}C and 1400 rpm for 2 h. After 2 h a second addition of 20 µL of 2.0 mg/mL trypsin was added to each sample and they were placed back on the vortexer at 37^{0}C at a speed of 1400 rpm. The tryptic digestion was stopped after 20 h by the addition of 3 µL of 88% formic acid and samples were stored at 4°C until solid phase extraction (SPE) was performed.

SPE was utilized to remove non-volatile salts and other contaminants that could potentially interfere with ESI-MS analysis. Acidified DBS tryptic digests were brought to room temperature and centrifuged at 16k x *g* for 10 min. SPE was carried out using Water's Oasis MCX 30 g 96-well plates on a Biotage 96+ Positive Pressure Manifold flowing nitrogen gas. Resin was initially conditioned by applying 1 mL methanol at 2 PSI, then rinsing with 1 mL 2.8% ammonium hydroxide in water at 3 PSI. The resin was

then flushed with 2 mL of methanol at 2 PSI, followed by rinsing with 3 mL of 0.1% formic acid in water at 9 PSI. 1.25 mL of acidified DBS tryptic digest was loaded on the acidified resin at 2 PSI. Salts were washed using 1 mL of 0.1% FA in water, followed by a neutrals wash with 0.1% FA in methanol. Finally, analytes were eluted with 1 mL of 2.8% ammonium hydroxide in methanol into a 1.5 mL 96-well plate at 0.5 PSI, with an increase to 3 PSI to elute all solvent off the resin. The eluent was transferred to a 1.5 mL Low Bind microfuge tube (Fisher Scientific) and subsequently de-solvated in a centrifuge under vacuum. Prior to analysis, the samples were re-suspended in 250 µL of 95:5 H_2O/acetonitrile/0.2% TFA and vortexed at 1400 rpm at RT for 2 h on a thermomixer. After vortexing, the samples were further diluted with 250 µL of a 0.2% TFA solution, for a total dilution of 1:300 from the approximate volume of blood (~3 µL) on the DBS. Prior to injection, samples were further diluted 1:2 in 98:2 H_2O/acetonitrile/0.2% TFA. Plasma digests were re-suspended in 750 µL of 95:5 H_2O/acetonitrile/0.2% TFA and vortexed at 1400 rpm at RT for 2 h on a thermomixer. After vortexing, the samples were further diluted with 750 µL of a 0.2% TFA solution. Prior to injection, samples were further diluted 1:3 in 98:2 H_2O/acetonitrile/0.2% TFA.

Preparation of dried blood spots for assay development and provisional validation

Standard D consisted of a red cell pool from left over samples with HbA1c values greater than 12% and a serum pool from left over samples with LDL-C concentration of >150 mg/dL. To avoid agglutination of the pooled standard D, serum from the high HbA1c whole blood samples was removed via centrifugation (5 min at 900 x g) and the RBCs were washed four times with cold (4^0C) 0.9% (m/v) sodium chloride (Baxter Healthcare Corporation); samples were stored on ice between wash steps. The volume of serum that was removed from each sample was replaced with the pooled high LDL-C serum and gently mixed by rotation for 5 min. Each sample was inspected via microscopy for agglutination and none was observed. These samples were then combined, gently mixed via rotation for 5 min and inspected for agglutination. Again, none was observed. Approximately 3 mL of the pooled Standard D was removed to measure HbA1c, HDL, LDL, triglycerides, cholesterol, apoA-I and apoB as described above. Standard D was flash frozen in a dry ice/acetone bath and subsequently transferred to -80^0C. After 24 h at -80°C, Standard D was allowed to thaw; then vortexed for 1 min to ensure complete lysis of red blood cells and spotted as described above. Preparation of standard H utilized a single healthy donor. A serum or whole blood aliquot of Standard H used to measure apoA-I and apoB concentrations, as well as HDL-C, LDL-C, cholesterol and triglycerides. The Standard H was frozen for 24 h at -80°C; then thawed and vortexed for 1 min to ensure complete lysis of red blood cells and spotted as described above.

Trypsin digestion kinetics of apoA-I, apoB and HbA1c in DBS were qualitatively assessed by time course analysis using Standard H in triplicate using the digestion protocol outlined above. Aliquots of 100 µL were taken at 0, 1, 2, 3, 5, 7, 9, 12, 20, 22 and 24 h and each digestion aliquot was stopped using 1 µL of 88% formic acid and placed at 4°C. After all time points were collected, SPE was performed on each aliquot as outlined above using Water's Oasis MCX 2 g µElution 96-well plates. The time-point samples were then dried down, re-suspended and analyzed using nLC-PRM-MS as outlined below.

A single point calibrator (SPC) was prepared using a 1:1 mix of Standard H and Standard D and spotted as described above. Assay reproducibility was evaluated using a 5 x 5 procedure, such that 5 standard H and 5 standard D, 3.2 mm punches were extracted, digested, and analyzed individually each day over a 5-day period to determine intra- and inter-assay variability, which includes variability between 3.2mm punches due to distribution of analytes within a DBS (chromatographic effects) and potential variability between spots due to spotting technique. To assess the linear response of this method, three dilutions of standard H

and D whole blood were admixed 3:1 (H-to-D), 1:1, and 1:3 and spotted on Whatman 903 paper as described above. Lower limit of quantification (LLOQ) was determined by analyzing dilutions of standard H and D whole blood in chicken whole blood (Pel-Freez Biologicals) at the following dilutions: 1:1, 1:2, 1:4, 1:6, 1:8 and 1:10 of standard H or D whole blood to chicken whole blood.

To assess the potential effect of interferences on the accuracy and precision of the assay, solutions containing triglyceride-rich lipoproteins, enriched in albumins and γ-globulins (total protein), hemolyzed RBCs and conjugated and unconjugated bilirubin were spiked (Sun Diagnostics) into aliquots of standard H and the mixtures were spotted as described above. Thermal stability of the DBS was determined by storing DBS with silica-based desiccant at 37^0C, 25^0C, 4^0C and -20^0C for 14 days and comparing peak areas and peak area ratios of peptides to spots stored at -80^0C using single factor ANOVA. Stability of peptides in solution was determined by re-injecting samples after 48 hours on the autosampler at 7^0C. Finally, the correlation of plasma apoA-I, apoB, and HbA1c was analyzed using matched EDTA-whole blood DBS and capillary DBS obtained from 36 human subjects (**Supplemental Table 1**).

Nano-LC-parallel reaction-mass spectrometry

Peptides were separated using reversed-phase chromatography on an Easy nLC-1000 liquid chromatography/auto sampler system (Thermo Fisher Scientific). The trapping and analytical capillary columns were prepared in-house using 3 cm x 150 µm i.d. and 15 cm x 75 µm i.d. fused silica capillary glass, respectively (Polymicro Technologies). Trapping columns were prepared with Kasil frits [1] and packed with 4 µm C12 resin (Jupiter Proteo, Phenomenex, Torrance, CA). Integrated analytical columns and emitters were prepared using a Sutter Instrument Co. Model P-2000 laser-based micropipette puller (Sutter Instrument Co.) and packed with 5 µm C18 resin (Magic C18AQ, Michrom BioResources). Mobile phase A was 98:2 water/acetonitrile/0.1% FA and mobile phase B was 98:2 acetonitrile/water/0.1% FA. Sample injection volumes were 3 µL with a 6 µL trapping column wash at a flow rate of ~2.5 µL/min. Following sample injection, the needle assembly and injection loop were washed twice with IPA/0.1% FA, then four times with 0.1% FA. The gradient scheme was as follows (time, %B, flow rate): 0.0 min, 2%, 500 nL/min; 12 min, 40%, 500 nL/min; 15 min, 60%, 500 nL/min, 15.5 min, 80%, 500 nL/min, 17.5 min, 80%, 900 nL/min; 18 min, 2%, 900 nL/min; 25 min, 2%, 500 nL/min; 25.5 min, 2%, 500 nL/min; 27 min (**Supplemental Table 2**).

The liquid chromatography eluent was directed on-line to a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific) equipped with a custom-built nanospray ionization source operating in positive ion detection mode at an electric potential difference of +2.2k volts, capillary temperature of 275°C and S-lens RF level of 70. MS^2 instrument resolution was set at 17,500 with an isolation window of 1.0 m/z and data was collected in profile mode with normalized collision energy (NCE) of 27 for all precursor ions. Ion current monitoring was performed using a microscan setting of 1, with an AGC target of $1.0x10^6$ ions and maximum injection time of 70 ms. A polysiloxane background ion of 445.12002 m/z was used as a lock mass (**Supplemental Table 3)**. An inclusion list containing m/z values for protonated precursor peptide ions of interest was generated in Skyline-daily (Ver. 3.1.1.8663) [2] using FASTA files from UniProtKB/Swis-Prot database [3]. Two-minute wide chromatographic windows were included in the inclusion list based on iRT retention time [4] values using eight peptides from ^{15}N-apoA-I and imported into Xcalibur using .csv format file for scheduled nanoLC-PRM analysis of peptides from the tryptic digestion of apoA-I, apoB and HbA1c in DBS.

References

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Supplementary Table 1. Plasma lipid and blood sugar values for calibrators and correlation samples[a].

Sample **Number**	**Sample ID**	**HbA1c[a]**	**ApoA -I[b]**	**ApoB[b]**	**Cholesterol[b]**	**HDL-C[b]**	**Direct LDL-C[b]**	**Triglycerides[b]**	**Non-HDL-C[b]**
1	Standard H	5.8	266	92	235	98	114	113	137
2	Standard D	14.1	98	115	194	30	142	108	164
3	131001	5.2	159	82	186	57	102	78	129
4	131002	4.9	133	49	127	50	58	61	77
5	131003	5.0	165	69	171	63	81	58	108
6	131004	4.6	139	104	203	45	132	117	158
7	131005	4.9	157	60	148	52	68	129	96
8	131006	5.2	210	95	191	63	91	253	128
9	131007	5.3	231	75	222	106	82	82	116
10	131008	6.8	114	140	232	31	148	209	201
11	131009	5.5	219	92	223	71	113	172	152
12	131010	6.6	196	118	226	53	132	242	173
13	131011	4.7	128	76	149	43	90	66	106
14	131012	4.7	169	88	175	49	109	60	126
15	131013	5.2	168	106	222	51	132	151	171
16	131014	4.7	127	102	196	34	102	346	162
17	131015	5.9	115	93	160	30	113	104	130
18	131016	4.6	134	77	155	44	97	54	111
19	131017	5.4	139	75	168	48	98	62	120
20	131018	4.8	160	90	203	59	116	80	144
21	131019	5.3	157	89	184	53	106	108	131
22	131020	5.2	148	95	188	43	114	145	145
23	131021	5.4	135	114	203	39	143	109	164
24	131022	5.1	156	53	143	62	63	48	81
25	131023	5.2	141	60	142	57	67	53	85
26	131024	5.0	126	110	199	37	130	190	162
27	131025	5.3	127	123	216	36	149	136	180
28	131031	5.4	160	63	156	58	74	59	98
29	131032	5.6	155	71	186	65	101	53	121
30	131033	5.3	150	111	214	47	135	105	167
31	131034	9.9	190	86	199	70	108	53	129
32	131035	5.8	124	104	188	35	124	142	153
33	131036	6.0	147	106	197	37	117	258	160
34	131037	6.2	139	58	127	49	59	25	78
35	131038	6.2	163	99	204	53	129	88	151
36	131039	5.1	137	108	199	43	131	178	156
37	131040	5.3	123	64	139	41	82	83	98
38	131041	6.6	175	66	189	74	97	41	115

[a]Concentration unit as Percent HbA1c (%HbA1c)

[b]Concentration units of mg/dL

Supplementary Table 2. Chromatographic schedule for nanoflow LC-PRM analysis

Time (min)	Flow (nL/min)	%A[a]	%B[a]	Gradient Curve[b]
0.0	500	98	2	0
12.0	500	60	40	6
15.0	500	40	60	6
15.5	500	20	80	6
17.5	900	20	80	11
18.0	900	98	2	6
25.0	900	98	2	11
25.5	500	98	2	11
27.0	500	98	2	11

[a]Mobile phase A was 98% H_2O/2% acetonitrile/0.1%FA and mobile phase B was 98% acetonitrile/2% H_2O/0.1% FA.
[b]Curve gradient codes are as follows: 0, initial conditions; 6, linear gradient from previous condition to current; 11, hold condition constant from previous condition to the current time, changing to new conditions in a step function.

Supplementary Table 3. Parallel Reaction Monitoring MS^2 Parameters

Resolution @ 200 m/z	AGC* Target	Maximum IT* (ms)	Isolation Window Width (m/z)	NCE*
17,500	1.0e6	70	1.0	27

*****AGC: Automatic Gain Control; IT: Injection Time; NCE: Normalized Collision Energy

Supplementary Table 4. Average Absolute Difference of Quantification Peptide Transition Ion Ratios[a].

Hemoglobin A1c		Apolipoprotein A-I						Apolipoprotein B			
VHLTPEEK		DLATVYVDVLK			VQPYLDDFQK			GFEPTLEALFGK		VSALLTPAEQTGTWK	
Unglycated	Glycated	Endogenous	IS_pep	IS_prot	Endogenous	IS_pep	IS_prot	Endogenous	IS_pep	Endogenous	IS_pep
0.011	0.011	0.014	0.023	0.020	0.020	0.024	0.027	0.040	0.019	0.042	0.014

[a]Ratio calculated using two most abundant transition ions for each peptide.

Supplementary Table 5. Peptides used for quantification and iRT calibration

				Fragment Ion m/z[b]									
Protein	**Purpose**	**Peptide[a]**	**Precursor m/z**	**y12, 1+**	**y11, 1+**	**y10, 1+**	**y9, 1+**	**y8, 1+**	**y7, 1+**	**y6, 1+**	**y5, 1+**	**y4, 1+**	**y3, 1+**	**y2, 1+**
ApoA-I	Quantifying	DLATVYVDVLK	618.35				1007.58	936.54	835.49	736.42	573.36	474.29	359.27	260.20
ApoA-I*	Normalization	DLATVYVDVL**K**	622.36				1015.59	944.55	843.51	744.44	581.37	482.31	367.28	268.21
ApoA-I#	Normalization/iRT	***DLATVYVDVLK***	624.33				1017.55	945.51	843.47	743.40	579.34	479.28	363.25	263.19
ApoA-I	Quantifying	VQPYLDDFQK	626.81					1025.49	928.44	765.38	652.29	537.27	422.24	275.17
ApoA-I*	Normalization	VQPYLDDFQ**K**	630.82					1033.51	936.46	773.39	660.31	545.28	430.25	283.19
ApoA-I#	Normalization/iRT	***VQPYLDDFQK***	633.30					1035.46	937.41	773.35	659.27	543.25	427.22	279.16
ApoA-I	Normalization	DYVSQFEGSALGK	700.84		1122.58	1023.51	936.48	808.42	532.31	475.29	388.26	317.22	204.13	
ApoA-I#	Normalization/iRT	***DYVSQFEGSALGK***	708.32		1135.54	1035.47	947.45	817.39	539.29	481.27	393.24	321.21	207.13	
ApoA-I#	iRT Calibration	***LLDNWDSVTSTFSK***	815.37	1401.57	1285.55	1169.51	981.44	865.41	777.39	677.32	575.28	487.25	385.20	237.14
ApoA-I#	iRT Calibration	***LAEYHAK***	421.21								655.29†	525.25†	361.19†	221.14†
ApoA-I#	iRT Calibration	***ATEHLSTLSEK***	615.29				1055.50	925.46	785.41	671.33	583.30	481.26	367.18	279.15
ApoA-I#	iRT Calibration	***QGLLPVLESFK***	622.34				1055.60	941.52	827.44	729.39	629.32	515.24	385.20	297.17
ApoA-I#	iRT Calibration	***VSFLSALEEYTK***	700.34			1211.58	1063.52	949.44	861.41	789.37	675.29	545.25	415.21	251.15
ApoB	Quantifying	ATFQTPDFIVPLTDLR	917.49		1285.72	1188.66	1073.64	926.57	813.48	714.41	617.36	504.28	403.23	
ApoB*	Normalization	ATFQTPDFIVPLTDL**R**	922.50		1295.72	1198.67	1083.64	936.58	823.49	724.42	627.37	514.29	413.24	
ApoB	Quantifying	SVSDGIAALDLNAVANK	829.44			1028.57	957.54	844.45	729.43	616.34	502.30	431.26		
ApoB*	Normalization	SVSDGIAALDLNAVAN**K**	833.45			1036.59	965.55	852.47	737.44	624.36	510.31	439.28		
ApoB	Quantifying	IADFELPTIIVPEQTIEIPSIK	1233.69	1353.76	1254.69	1028.60	900.54	686.41	557.37	444.28				
ApoB*	Normalization	IADFELPTIIVPEQTIEIPSI**K**	1237.70	1361.78	1662.71	1036.61	908.55	694.42	565.38	452.30				
ApoB	Quantifying	VSALLTPAEQTGTWK	801.43	1344.72	1231.63	1118.55	1017.50	920.45	849.41	720.37	592.31	491.26	434.24	
ApoB*	Normalization	VSALLTPAEQTGTW**K**	805.44	1352.73	1239.65	1126.56	1025.51	928.46	857.42	728.38	600.32	499.28	442.25	
ApoB	Quantifying	GFEPTLEALFGK	654.85			1104.59	975.55	878.50	777.45	664.37	535.32	464.29		
ApoB*	Normalization	GFEPTLEALFG**K**	658.85			1112.61	983.57	886.51	785.46	672.38	543.34	472.30		
ApoB	Quantifying	VHLTPEEK	476.76							716.38	603.30	502.25	405.20	276.16
ApoB	Quantifying	Gly_VHLTPEEK	557.79							716.38	603.30	502.25	405.20	276.16

[a] ^{13}C and ^{15}N heavy labeled lysine and arginine residues are in bold. ^{15}N labeled amino acid residues are in bold and italics.

[b] Transition ion designated as: y-ion fragment, ion charge state.

†The 2+ charge state transition ion was also monitored.

* Stable isotope labeled peptide incorporating amino acids with both ^{13}C and ^{15}N labeled lysine and arginine residues at the C-terminus.

Peptide derived from stable isotope labeled protein where ^{15}N labels have been incorporated into all amino acid residues.

Supplementary Table 6. Comparison of precision study data without and with calibrators using IS_prot

	Without Calibrator						With Calibrator					
	Healthy		Diseased		Healthy	Diseased	Healthy		Diseased		Healthy	Diseased
	CV_{Intra}	CV_{Inter}	CV_{Intra}	CV_{Inter}	CV_{Tot}	CV_{Tot}	CV_{Intra}	CV_{Inter}	CV_{Intra}	CV_{Inter}	CV_{Tot}	CV_{Tot}
ApoA-I[a]	6%	9%	6%	8%	10%	10%	6%	7%	6%	6%	9%	8%
ApoB[a]	10%	30%	15%	20%	32%	25%	10%	14%	12%	13%	17%	18%

[a]Peak area of endogenous peptide signal normalized by IS_pep peak area.

Supplementary Table 7. Linearity studies[a].

Percent HbA1c	Ratio[b]	ApoA-I (mg/dL)	IS_pep	IS_prot	ApoB (mg/dL)	IS_pep	IS_prot
5.8	0.0% (6%)	266	0.0% (6%)	-0.4% (8%)	92.0	0.0% (3%)	-0.6% (9%)
7.9	-7.3% (4%)	224	1.3% (4%)	2.5% (7%)	97.8	2.2% (4%)	-1.3% (11%)
10.0	0.2% (4%)	182	9.3% (9%)	10.4% (3%)	103.5	-5.2% (5%)	7.5% (4%)
12.0	5.3% (8%)	140	8.0% (3%)	14% (1%)	109.3	-6.5% (5%)	19.7% (5%)
14.1	1.3% (9%)	98	19.4% (9%)	19.2% (5%)	115.0	1.1% (4%)	13.9% (3%)

[a]Linearity was assessed by mixing Standard H and D in defined ratios. The bias of each is denoted with the %CV of replicate measurements in parentheses.

[b]The percent HbA1c determined by nLC-PRM-MS was calculated as the ratio of the peak area of glycated HBB peptide (gly_VHLTPEEK) to the sum of the glycated and non-glycated HBB peptides.

Supplementary Table 8. Lower limit of quantification of Standard H[a].

ApoA-I (mg/dL)	IS_pep	IS_prot	ApoB (mg/dL)	IS_pep	IS_prot
133.0	91% (10%)	110% (7%)	46.0	101% (6%)	114% (7%)
87.8	83% (5%)	106% (0%)	30.4	96% (5%)	123% (11%)
53.2	81% (2%)	109% (1%)	18.4	99% (10%)	129% (6%)
38.0	80% (4%)	113% (9%)	13.2	88% (7%)	143% (20%)
29.6	81% (6%)	109% (9%)	10.2	91% (20%)	123% (13%)
23.9	79% (10%)	116% (7%)	8.3	73% (12%)	101% (20%)
0.0	NA (NA)	NA (NA)	0.0	NA (NA)	NA (NA)

[a]The lower limit of quantification was assessed by mixing Standard H with EDTA-whole blood from chicken (Gallus gallus). The percent recovery is denoted with the %CV of replicate measurements in parentheses.

Supplementary Table 9. Lower limit of quantification of Standard D[a].

ApoA-I (mg/dL)	IS_pep	IS_prot	ApoB (mg/dL)	IS_pep	IS_prot
49.0	95% (13%)	108% (9%)	57.5	108% (10%)	106% (9%)
32.3	92% (6%)	104% (5%)	38.3	104% (6%)	106% (17%)
19.6	95% (8%)	115% (6%)	23.0	119% (4%)	122% (10%)
14.0	75% (6%)	92% (4%)	16.4	89% (9%)	83% (9%)
10.9	80% (7%)	101% (8%)	12.8	93% (10%)	101% (23%)
8.8	76% (13%)	93% (18%)	10.5	91% (15%)	93% (12%)
0.0	NA (NA)	NA (NA)	0.0	NA (NA)	NA (NA)

[a]The lower limit of quantification was assessed by mixing Standard B with EDTA-whole blood from chicken (Gallus gallus). The percent recovery is denoted with the %CV of replicate measurements in parentheses.

Supplementary Table 10. Interference studies[a].

Protein	Triglycerides (>1000 mg/dL)	High Protein (>8 g/dL)	Hemolysis (>500 mg/dL)	Conjugated Bilirubin (>20 mg/dL)	Unconjugated Bilirubin (>20 mg/dL)
HbA1c	101% (13%)	115% (7%)	96% (10%)	94% (9%)	103% (27%)
ApoA-1; IS_{pep}	97% (3%)	120% (4%)	109% (10%)	89% (8%)	98% (7%)
ApoB; IS_{pep}	300% (18%)	122% (6%)	109% (3%)	101% (11%)	99% (15%)
ApoA-1; IS_{prot}	99% (14%)	117% (9%)	108% (4%)	99% (7%)	108% (7%)
ApoB; IS_{prot}	275% (18%)	110% (10%)	106% (22%)	105% (6%)	108% (18%)

[a]Potential interfering substances were assessed by spiking standard interferents obtained from Sun Diagnostics in Standard A. The percent recovery is denoted with the %CV of replicate measurements in parentheses.

Supplementary Table 11. Temperature stability of dried blood spots[a].

Protein	-80^{o}C	-20^{o}C	4^{o}C	25^{o}C	37^{o}C
HbA1c	5%	8%	10%	9%	4%
ApoA-1; IS_{pep}	4%	10%	11%	5%	2%
ApoB; IS_{pep}	14%	11%	6%	7%	10%
ApoA-1; IS_{prot}	2%	13%	9%	7%	6%
ApoB; IS_{prot}	10%	15%	18%	22%	14%

[a]Dried blood spots were incubated at the specified temperature in sealed bags containing silica-based dessicant for 14 days to determine thermal stability as denoted by the %CV of replicate measurements.

Supplementary Table 12. Comparison of storage temperatures for dried blood spots[a].

Protein	P-value	P-value (37^{o}C Removed)
HbA1c	0.240	0.240
ApoA-1; IS_{pep}	0.050	0.958
ApoB; IS_{pep}	0.009	0.398
ApoA-1; IS_{prot}	0.243	0.962
ApoB; IS_{prot}	0.777	0.886

[a]Determined using Single Factor ANOVA

Supplementary Table 13. Stability of DBS samples after 48 hours at 7^0C[a].

Protein	Standard H	1:1, Std H:Std D	Standard D
HbA1c	8% (0.955)	4% (0.285)	10% (0.486)
ApoA-1; IS_{pep}	10% (0.502)	5% (0.880)	5% (0.781)
ApoB; IS_{pep}	11% (0.745)	7% (0.464)	6% (0.544)
ApoA-1; IS_{prot}	8% (0.437)	4% (0.560)	7% (0.611)
ApoB; IS_{prot}	14% (0.080)	12% (0.207)	9% (0.101)

[a]Resuspended DBS samples were analyzed and left on the auto-sampler at 7^0C for 48 h, then re-analyzed. The %CV of replicate measurements is denoted and comparison, by two-tailed t-test, of the analyses is in parentheses.

Supplementary Table 14. Correlation experiments normalized using IS_pep[a].

	Capillary	Whole Blood	Plasma
HbA1c[b]	0.858 (11%)	0.845 (9%)	
ApoA-I[b]	0.822 (12%)	0.783 (17%)	0.818 (12%)
HDL-C	0.825	0.726	0.834
ApoB[b]	0.830 (12%)	0.809 (16%)	0.974 (4.5%)
LDL-C	0.829	0.800	0.730
Non-HDL-C	0.819	0.799	0.796

[a]The correlation coefficients comparing traditional clinical methodologies and nLC-PRM-MS were determined to assess the relationship between the different assays. Values represent the Pearson product-moment correlation coefficient from single measurements of 36 human DBS and 29 matched plasma normalized using IS_pep.

[b]Mean percent bias of measurements using LC-PRM-MS with clinically validated assays as reference concentration from nephelometry or HPLC-UV represented in parenthesis.

Supplementary Table 15. Correlation experiments normalized using IS_prot[a].

	Capillary	Whole Blood	Plasma
HbA1c[b]	0.858 (11%)	0.845 (9%)	
ApoA-I[b]	0.871 (12%)	0.834 (14%)	0.924 (6%)
HDL-C	0.807	0.708	0.775
ApoB[b]	0.880 (16%)	0.850 (14%)	0.817 (23.9%)
LDL-C	0.853	0.880	0.597
Non-HDL-C	0.835	0.830	0.660

[a]The correlation coefficients comparing traditional clinical methodologies and nLC-PRM-MS were determined to assess the relationship between the different assays. Values represent the Pearson product-moment correlation coefficient from single measurements of 36 human DBS and 29 matched plasma normalized using IS_prot.

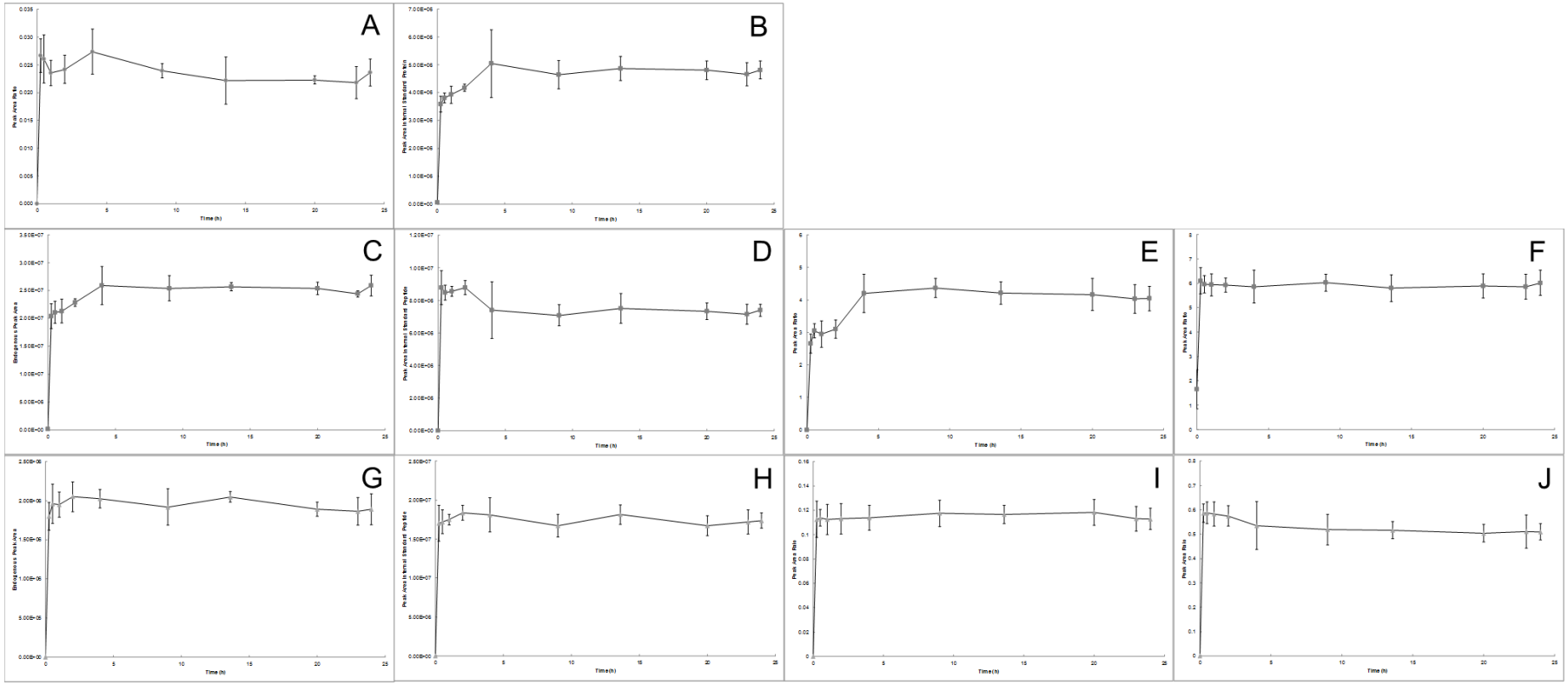
[b]Mean percent bias of measurements using LC-PRM-MS with clinically validated assays as reference concentration from nephelometry or HPLC-UV represented in parenthesis.

Supplementary Table 16. Correlation of capillary DBS and EDTA-WB DBS normalized using IS_pep and IS_prot[a].

Protein	Capillary vs WB
HbA1c	0.730
ApoA-1; IS_{pep}	0.784
ApoB; IS_{pep}	0.754
ApoA-1; IS_{prot}	0.882
ApoB; IS_{prot}	0.821

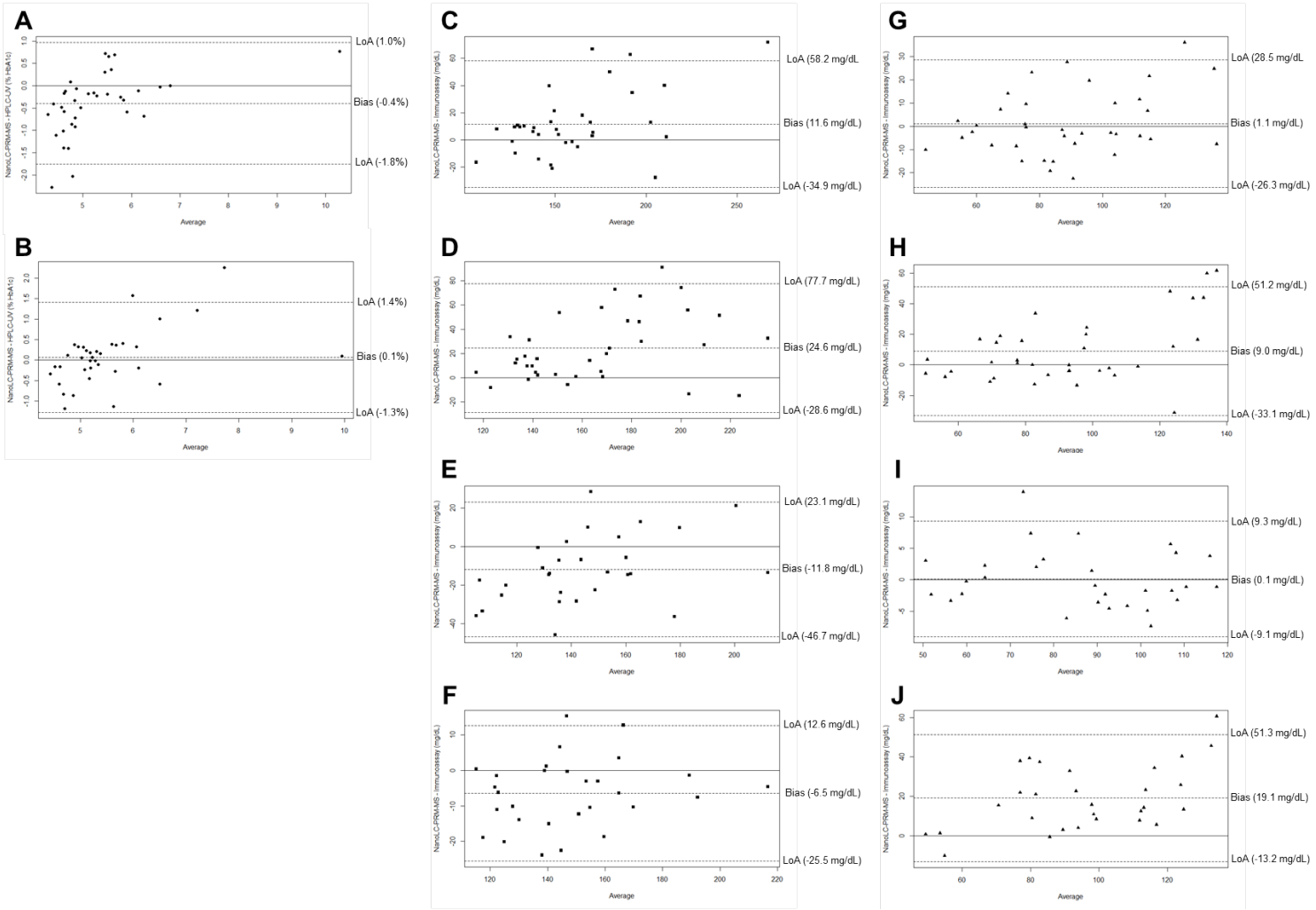
[a]The correlation coefficients comparing capillary and EDTA-WB DBS analyzed using nLC-PRM-MS to assess the relationship between the different assays. Values represent the Pearson product-moment correlation coefficient from single measurements of 36 human DBS normalized using IS_pep or IS_prot.

Supplementary Figure 1



Supplementary Figure 1. Digestion time-course for standard H. (**A**) Peak area ratio of glycated VHLTPEEK to the averaged peak areas of glycated VHLTPEEK and non-glycated VHLTPEEK (total Hb-β), (**B**) averaged peak areas of the ^{15}N-apoA-I peptides, (C) averaged peak area of the apoA-I endogenous peptides, (**D**) averaged peak area of the apoA-I internal standard peptides, (E) averaged peak area ratio of apoA-I normalized by IS_pep, (F) averaged peak area ratio of apoA-I normalized by IS_prot (**G**) averaged peak area of the apoB endogenous peptides, (**H**) averaged peak areas of the apoB internal standard peptides, (I) averaged peak area ratio of apoB normalized by IS_pep and (**F**) averaged peak area ratio of apoB normalized by IS_prot.

Supplementary Figure 2



Supplementary Figure 2. Agreement between the concentrations of HbA1c (●), apoA-I (■) and apoB (▲) measured using nLC-PRM-MS assay versus HPLC-UV or immunoassay were evaluated using difference (Bland-Altman) plots for %HbA1c from (**A**) capillary and (**B**) EDTA-WB DBS; apoA-I (mg/dL) from (**C**) capillary, (**D**) EDTA-WB DBS, (**E**) matched plasma (IS_pep) and (**F**) matched plasma (IS_prot) and apoB (mg/dL) (**G**) from capillary, (**H**) EDTA-WB DBS, (**I**) matched plasma (IS_pep) and (**J**) matched plasma (IS_prot). Bias and Limits of Agreement (LoA) were plotted as the concentration of the respective protein.