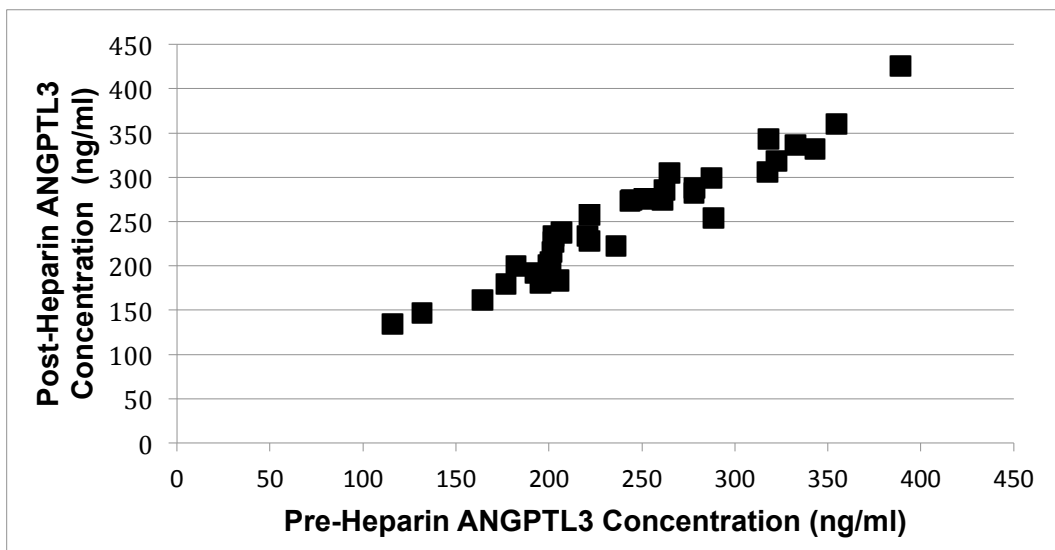


SUPPLEMENTAL MATERIAL

ANGPTL3 ELISA Validation

The Biovendor assay (Biovendor Laboratory Medicine, Prague, Czech Republic) was previously validated in published literature (Robciuc, M et al. JLR 2010, Stejskal, M, et al. Gen Physiol. Biophys 2007). Our internal validation consisted of running different positive controls. We obtained an inter-assay variability of 5.7%. Pre and post-heparinized plasma samples were compared to determine if heparin affects the ANGPTL3 concentrations. A pre and post-heparin analysis was done and the average net change between pre- and post- heparin was 4.6% (Figure I) ($R^2 = 0.93$).

Figure I. Comparison of ANGPTL3 Concentration in Pre-heparin and Post-heparin Samples



ANGPTL4 ELISA Validation

The ANGPTL4 ELISA validation involved testing different commercialized assay kits, which included: R & D DuoSet (R&D Systems, Minneapolis, MN), RayBio Human ANGPTL4 (RayBiotech, Inc., Norcross, GA) and Biovendor (Biovendor Laboratory, Prague, Czech Republic). For the R & D assay, the linearity in dilution (1x to 32x) was inconsistent with diluted samples having significantly lower ANGPTL4 concentrations.

The same finding was observed with the spike and recovery. Next, for the RayBio assay, the linearity of dilution was more consistent than the R & D assay. However, the spike and recovery for this assay was reduced (4%-50% recovery of spiked sample). Lastly, validation was performed with the Biovendor Assay. First, 30 healthy volunteers were tested twice for reproducibility and coefficient of variation ranged from 2% to 12% (outliers, 18% and 28%). Next, average recovery for spiked standard samples was 107%. Lastly, linearity of dilution (1x to 16x) resulted in a 1.5% - 6% coefficient of variation among the subjects. The final concentrations of the diluted samples were on average 82% of the expected value (based on the recombinant ANGPTL4 standard).

Table I. ANGPTL3 and ANGPTL4 Association with Lipid Parameters Adjusted for Demographics*

	ANGPTL3		ANGPTL4	
	β coefficient	P	β coefficient	P
Triglycerides, mg/dl	0.008	0.53	0.036	0.003
VLDL-C, mg/dl	0.005	0.82	0.037	0.149
LDL-C, mg/dl	3.65	1.93×10^{-5}	-3.77	2.01×10^{-5}
HDL-C, mg/dl	1.60	1.98×10^{-6}	-2.09	1.15×10^{-9}
Total cholesterol, mg/dl	5.74	1.04×10^{-8}	-4.45	1.79×10^{-5}
Apolipoprotein A-I, mg/dl	1.21	0.03	-1.93	0.002
Apolipoprotein A-II, mg/dl	0.17	0.27	-0.23	0.238
Apolipoprotein B, mg/dl	0.75	0.19	-2.43	1.6×10^{-4}
Apolipoprotein C-III, mg/dl	0.004	0.84	0.069	0.04

*Linear regression model adjusted for age, gender, and race. Beta coefficient indicates increment (positive or negative) in lipid parameter per 1-standard deviation increase in ANGPTL3 or log transformed ANGPTL4 concentrations. LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol. Triglycerides, VLDL-C, and Apolipoprotein C-III were log transformed.

Table II. ANGPTL3 and ANGPTL4 Association with Lipid Parameters Adjusted for Demographics and Statin Use*

	ANGPTL3		ANGPTL4	
	β coefficient	P	β coefficient	P
Triglycerides, mg/dl	0.01	0.445	0.04	0.008
LDL-C, mg/dl	2.07	0.009	-2.85	5.15×10^{-4}
HDL-C, mg/dl	1.52	6.78×10^{-6}	-2.02	4.1×10^{-9}
Total Cholesterol	4.07	1.77×10^{-5}	-3.44	4.18×10^{-4}

*Linear regression model adjusted for age, gender, race, and statin use. Beta coefficient indicates increment (positive or negative) in lipid parameter per 1-standard deviation increase in ANGPTL3 or log transformed ANGPTL4 concentrations. LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. Triglycerides were log transformed.

Table III. ANGPTL3 and ANGPTL4 Association with Metabolic Parameters*

	ANGPTL3		ANGPTL4	
	β coefficient	P	β coefficient	P
Body mass index, kg/m ²	0.65	1.3 x 10 ⁻⁵	1.54	2.0 x 10 ⁻¹⁶
Waist Circumference, inches	0.66	8.1 x 10 ⁻⁶	1.67	2.0 x 10 ⁻¹⁶
Blood Pressure, mmHg				
Systolic	-0.18	0.63	0.73	0.0205
Diastolic	-0.24	0.32	-0.67	0.014
Fasting Blood Glucose, mg/dl	-0.02	0.014	0.09	2.0 x 10 ⁻¹⁶
Hemoglobin A1C, %	0.04	0.33	0.48	2.0 x 10 ⁻¹⁶
Insulin, IU/ml	0.03	0.11	0.24	2.0 x 10 ⁻¹⁶
Free Fatty Acids, mEq/ml	0.004	0.81	0.15	6.4 x 10 ⁻¹⁴
Leptin, ng/ml	0.056	0.016	0.15	3.91 x 10 ⁻⁸
Adiponectin, mg/ml	0.064	0.003	-0.12	3.8 x 10 ⁻⁶

*Linear regression model adjusted for age, gender, and race. Beta coefficient indicates increment (positive or negative) in cardiometabolic outcome per 1-standard deviation increase in ANGPTL3 or log transformed ANGPTL4 concentrations. Fasting blood glucose, insulin, free fatty acids, leptin, and adiponectin were log transformed.