SUPPLEMENTAL MATERIALS

Heparin Does Not Regulate Circulating Human PCSK9 in a General Population

Vivian Q. Xia, BA[1,2]; Chui Mei Ong, BS[3,4]; Lucas S. Zier, MD, MS[1,2]; John S. MacGregor, MD, PhD[1,2]; Alan H. B. Wu, PhD[3,4]; John S. Chorba, MD*[,1,2]

Affiliations:
[1]Division of Cardiology, Zuckerberg San Francisco General Hospital
[2]Department of Medicine, University of California San Francisco
[3]Clinical Chemistry Laboratory, Zuckerberg San Francisco General Hospital
[4]Department of Laboratory Medicine, University of California San Francisco

Contents

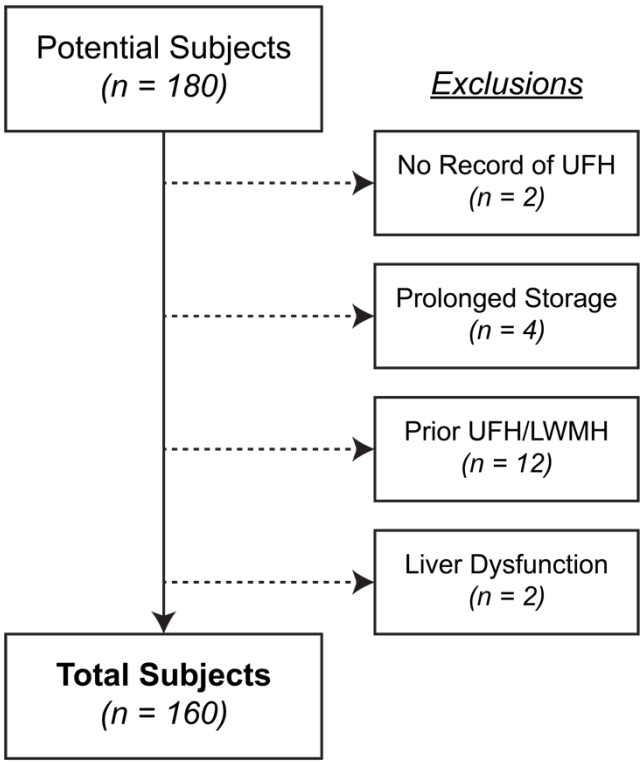

Figure S1: Flowchart of Subjects in Main Cohort. "Potential Subjects" indicates those in whom remnant blood samples were recovered and stored. "Total Subjects" indicates those remaining after exclusion criteria (right) were applied.

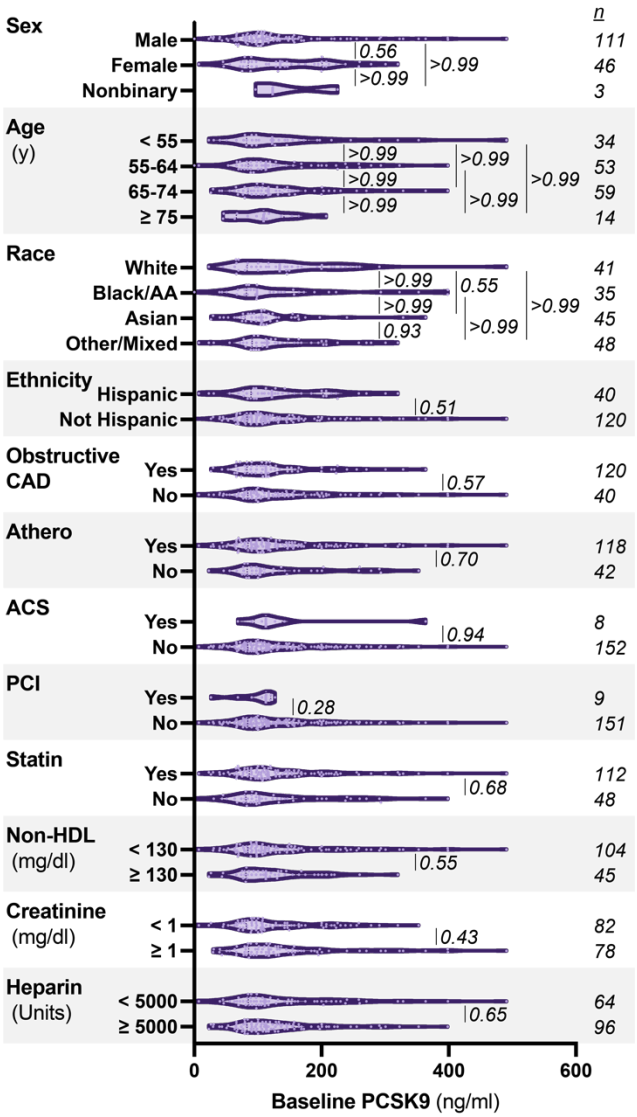

Figure S2: Baseline PCSK9 Concentrations of Subgroups of Main Cohort. Violin plots of the baseline (pre-UFH) plasma PCSK9 concentrations. *p* values represent Mann-Whitney tests for two groups, and Kruskal-Wallis test with Dunn's correction for three or more groups. n = number of subjects in the indicated stratum. AA = African American. CAD = coronary artery disease. Athero = atherosclerosis. ACS = acute coronary syndrome. PCI = percutaneous coronary intervention. HDL = high density lipoprotein.

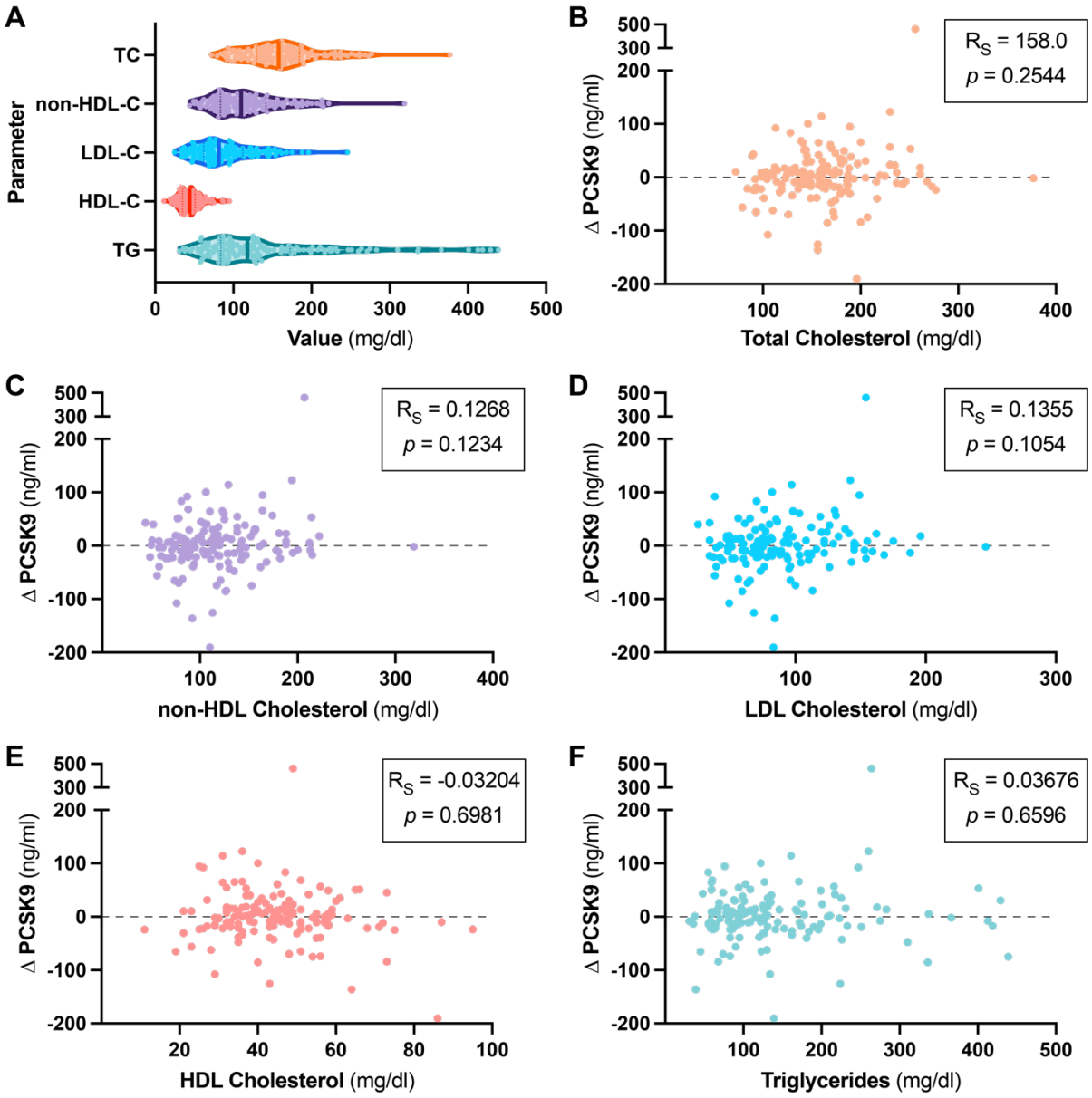

Figure S3: Lipid Panels of Study Subjects. *A)* Violin plots of the baseline lipid panels of the study subjects. Each dot represents a study subject. *B-F)* Plots of the change in PCSK9 due to UFH administration against pre-treatment plasma total cholesterol *(B)*, non-HDL cholesterol *(C)*, LDL cholesterol *(D)*, HDL cholesterol *(E)*, or triglycerides *(F)*. Spearman's correlation coefficient (R_S) and *p* value shown in box. Note the discontinuous Y axes in *B-F*. TC = total cholesterol. LDL = low density lipoprotein. HDL = high density lipoprotein. TG = triglycerides.

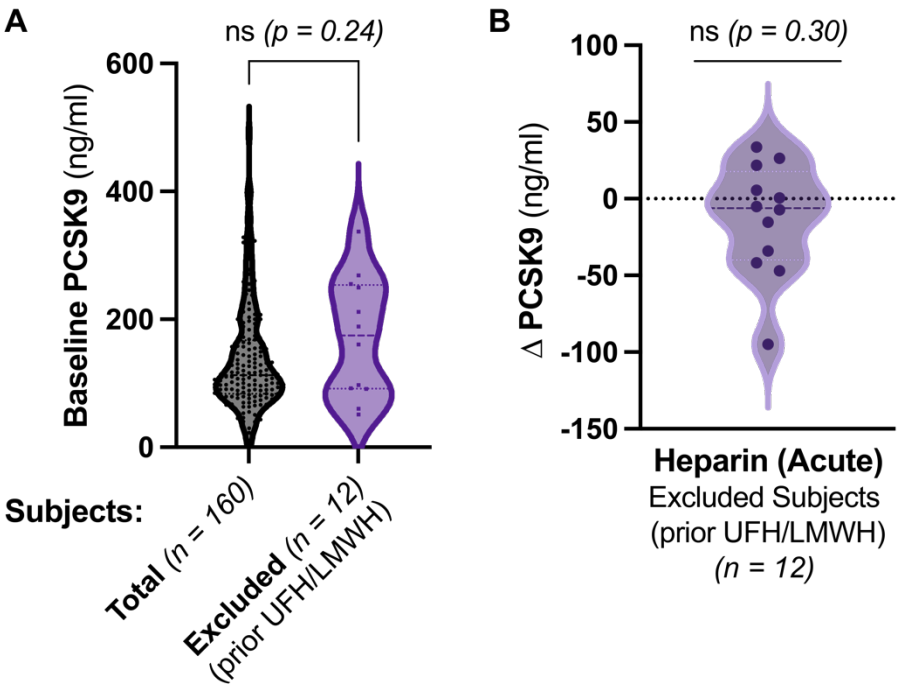

Figure S4: PCSK9 Concentrations in Patients Excluded Due to Prior Heparin Exposure. *A)* Baseline (pre-UFH) plasma PCSK9 in study subjects (after exclusions, black) and subjects excluded due to prior UFH or low-molecular weight heparin (LMWH) administration (purple). Mann-Whitney test shown. *B)* Difference in plasma PCSK9 before and after IV UFH in these excluded subjects. Wilcoxon signed-rank test, compared to a hypothetical median of 0, shown.

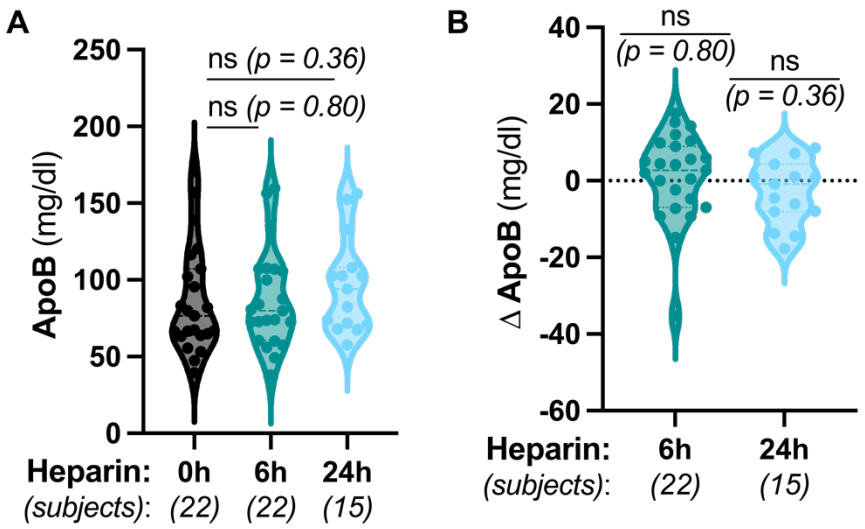

Figure S5: Effect of Heparin on Plasma ApoB. *A)* Violin plots of plasma apolipoprotein B (ApoB) concentrations before (black) or after 6 hr (teal) or 24 hr (light blue) infusions of IV UFH. Paired t test with Holm-Sidak correction shown. *B)* Violin plot of difference in plasma ApoB concentrations before and after IV UFH infusions. One sample t test (compared to hypothetical mean of 0) with Holm-Sidak correction shown. Source data is the same as from *A*.

Variable	*n*	*$\Delta PCSK9_{median}$ [ng/ml, (%)]*	*Raw p*	*Adj. p (in variable)*	*Adj. p (all strata)*
Sex					
Male	111	-1.72, (1.59)	0.82	0.96	>0.99
Female	46	3.72, (2.76)	0.80	0.96	>0.99
Nonbinary	3	40.5, (32.9)	0.25	0.58	>0.99
Age (y)					
< 55	34	-5.43, (-4.43)	0.57	0.83	>0.99
55-64	53	5.35, (4.75)	0.54	0.83	>0.99
65-74	59	-2.00, (-1.72)	0.45	0.83	>0.99
≥ 75	14	15.5, (14.2)	0.025	0.095	0.54
Race					
White	41	-0.306, (-0.228)	0.67	0.78	>0.99
Black/AA	35	-2.00, (-2.04)	0.53	0.78	>0.99
Asian	45	8.54, (8.08)	0.31	0.68	>0.99
Other/Mixed	48	3.42, (2.98)	0.15	0.47	0.99
Ethnicity					
Hispanic	40	3.42, (2.80)	0.25	0.44	>0.99
Not Hispanic	120	-1.86, (-1.66)	0.63	0.63	>0.99
Obstructive CAD					
Yes	64	3.06, (2.71)	0.29	0.50	>0.99
No	96	-2.05, (-1.75)	0.57	0.57	>0.99
Atherosclerosis					
Yes	118	-2.00, (-1.72)	0.77	0.77	>0.99
No	42	5.11, (4.94)	0.31	0.53	>0.99
ACS					
Yes	8	-8.00, (-7.09)	0.64	0.87	>0.99
No	152	-0.171, (-0.151)	0.77	0.87	>0.99
PCI					
Yes	9	7.25, (6.44)	0.36	0.59	>0.99
No	151	-0.377, (-0.331)	0.99	0.99	>0.99
Statin					
Yes	112	1.63, (1.42)	0.70	0.91	>0.99
No	48	-3.12, (-3.05)	0.90	0.91	>0.99
Non-HDL Chol					
< 130 mg/dl	104	-1.01, (-0.908)	0.61	0.61	>0.99
≥ 130 mg/dl	45	3.92, (3.48)	0.22	0.39	>0.99
Creatinine					
< 1 mg/dl	82	1.26, (1.18)	0.67	0.89	>0.99
≥ 1 mg/dl	78	-2.05, (-1.75)	0.97	0.97	>0.99
Heparin Dose					
< 5000 U	64	-3.80, (-3.43)	0.35	0.58	>0.99
≥ 5000 U	96	-0.341, (-0.301)	0.36	0.58	>0.99
Baseline PCSK9					
1st Quartile (low)	40	5.86, (8.76)	0.0014	0.0055	0.044
2nd Quartile	40	7.03, (7.18)	0.59	0.83	>0.99
3rd Quartile	40	-1.87, (-1.38)	0.63	0.83	>0.99
4th Quartile (high)	40	-31.6, (-13.9)	0.020	0.059	0.48

Table S1: Subgroup Analyses of Main Cohort. n = number of subjects in each stratum. $\Delta PCSK9_{median}$ denotes the difference between the observed median of the change in plasma PCSK9 from 0 (the null hypothesis). % $\Delta PCSK9_{median}$ normalizes the $\Delta PCSK9_{median}$ value to baseline plasma PCSK9 for that stratum. *p* values determined by the Wilcoxon matched-pairs signed rank test and adjusted by Holm-Sidak correction for either the strata within a given variable *(in variable)*, or all strata evaluated *(all strata)*.

Major Resources Table

Description	Source / Repository	Persistent ID / URL
PCSK9 ELISA kit	Abcam (ab209884)	https://www.abcam.com/human-pcsk9-elisa-kit-ab209884.html
PCSK9 ELISA kit	RayBiotech (ELH-PCSK9)	https://www.raybiotech.com/human-proprotein-convertase-9-pcsk9-elisa/
ApoB ELISA kit	MabTech (3715-1HP)	https://www.mabtech.com/products/elisa-pro-human-apob-3715-1hp
Prism v9	GraphPad	https://www.graphpad.com
Illustrator v22	Adobe	https://www.adobe.com/products/illustrator.html