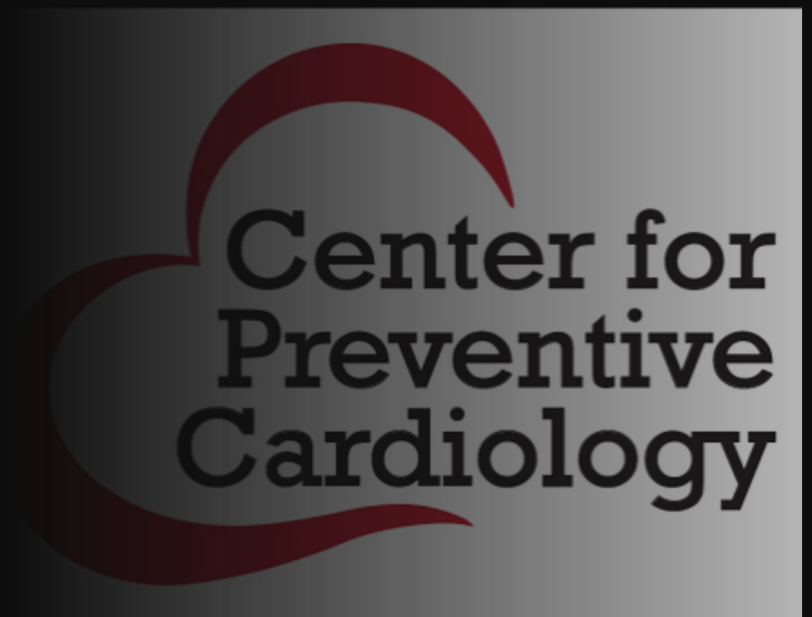
HDL known as the good cholesterol: Is it really good, is it bad, how do you tell?

Nathalie Pamir, PhD

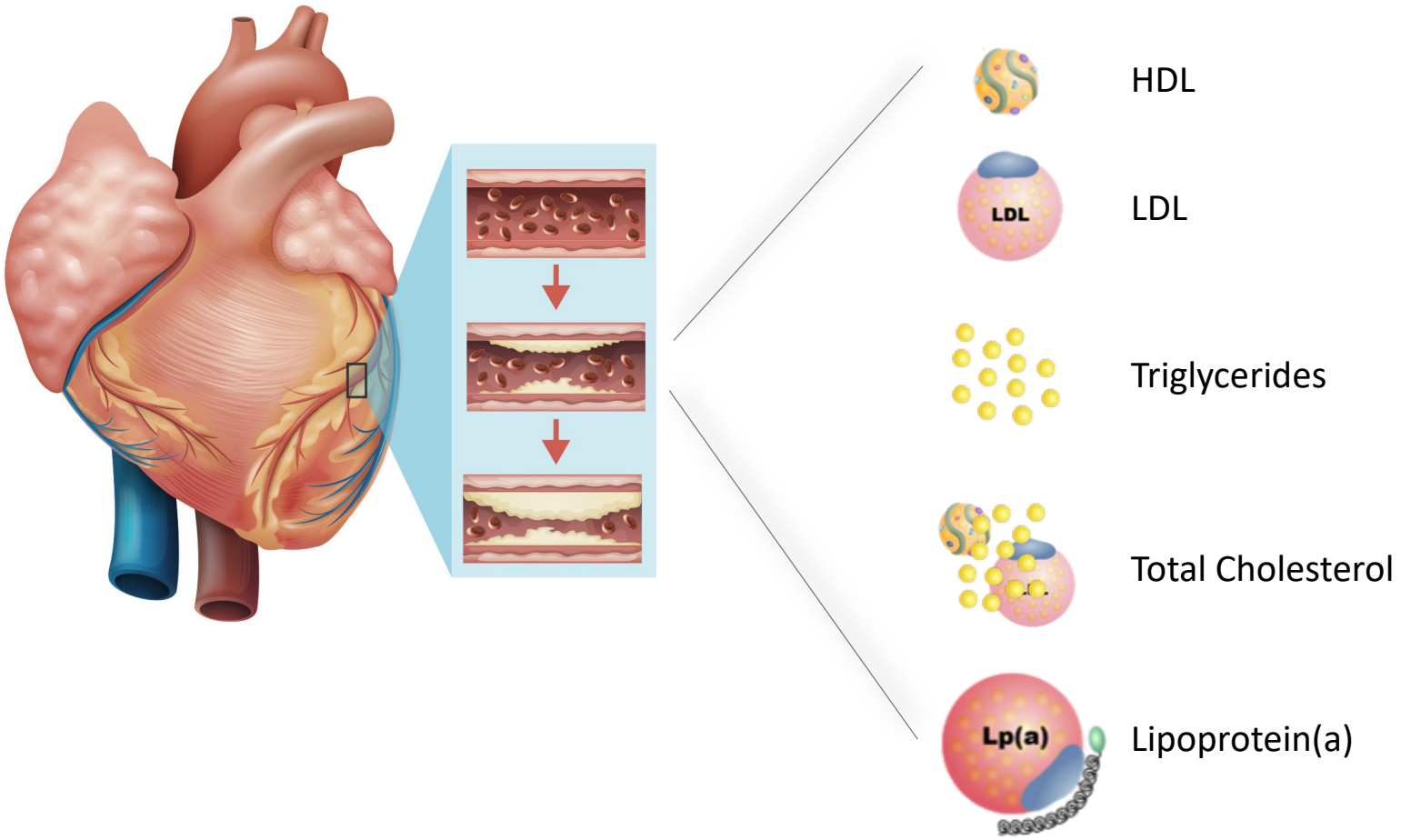
March, 2024



Life’s Essentials Eight

Donald M. Lloyd-Jones. Circulation. Life’s Essential 8: Updating and Enhancing the American Heart Association’s Construct of Cardiovascular Health: A Presidential Advisory From the American Heart Association, Volume: 146, Issue: 5, Pages: e18-e43, DOI: (10.1161/CIR.0000000000001078)

Heart Disease Prevention: Improved CVD Risk Prediction



Adapted from Harvard Health

Framingham Risk Score Calculator

Age		years
Sex	Female	Male
Smoker	No	Yes
Total cholesterol	Norm: 150 - 200	mg/dL ⇆
HDL cholesterol		mg/dL ⇆
Systolic BP	Norm: 100 - 120	mm Hg
Blood pressure being treated with medicines	No	Yes

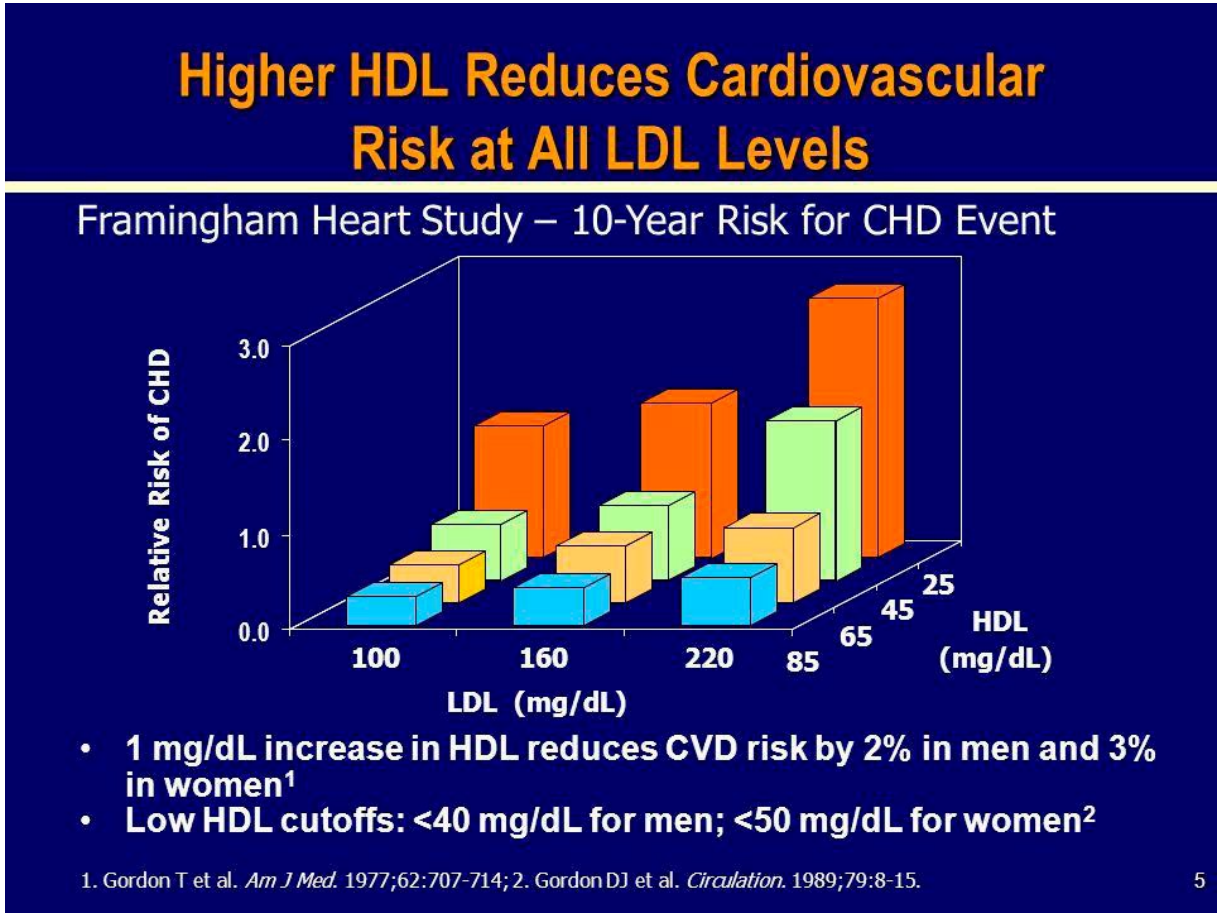
0.3 %
10-year risk of MI or death for this patient

2 %
Average 10-year risk of MI or death

Copy Results

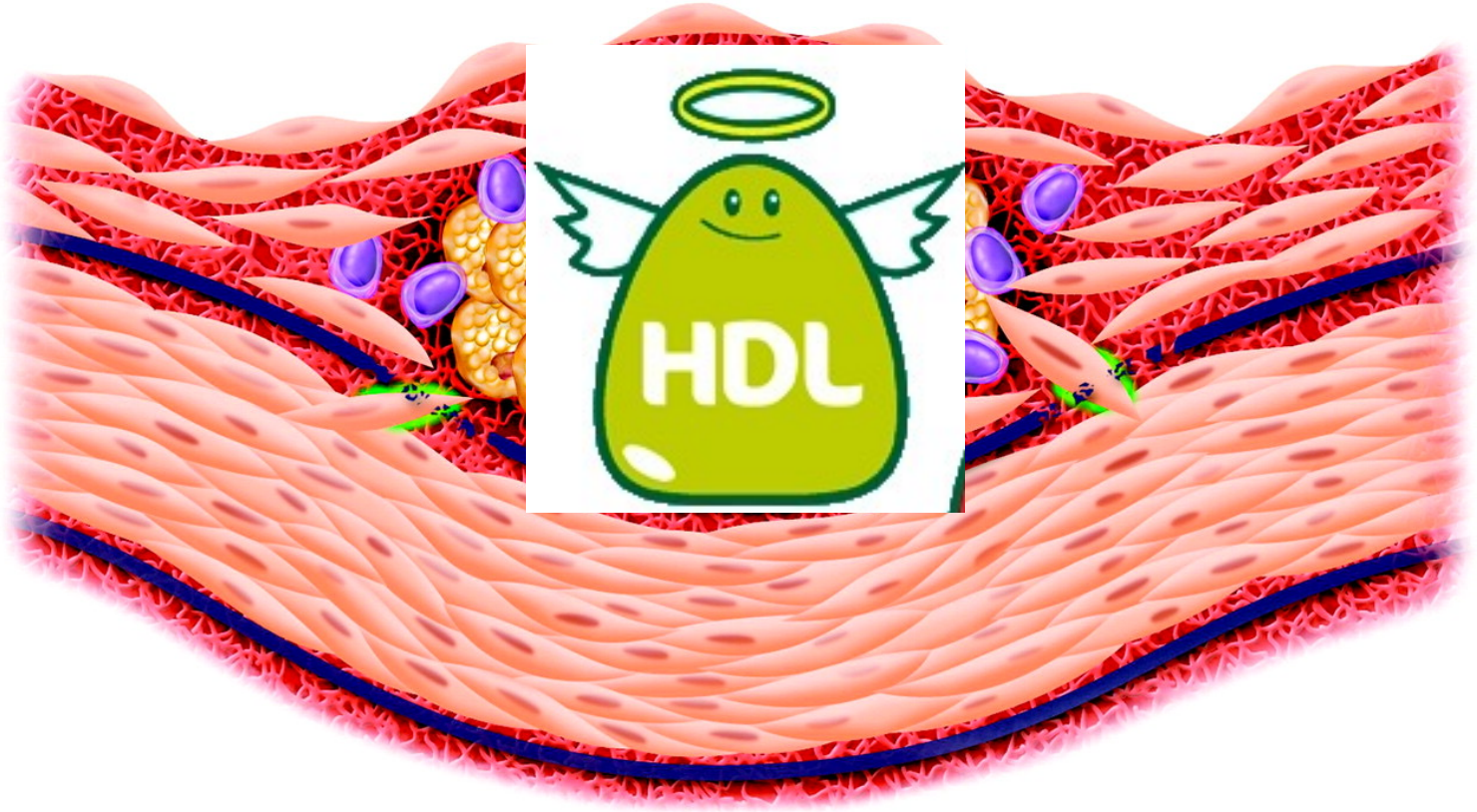
Next Steps »»

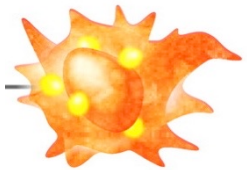
Low Levels of HDL-C Associate with Increased Risk of Coronary Artery Disease (CAD)



The study found high blood pressure and high blood cholesterol to be major risk factors for cardiovascular disease.

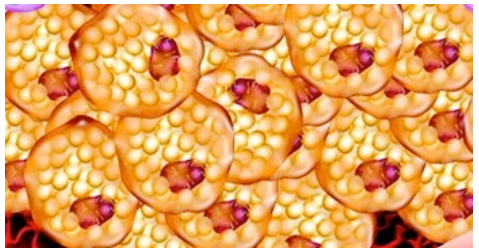
Gordon et al. *Am J Med*, 1977

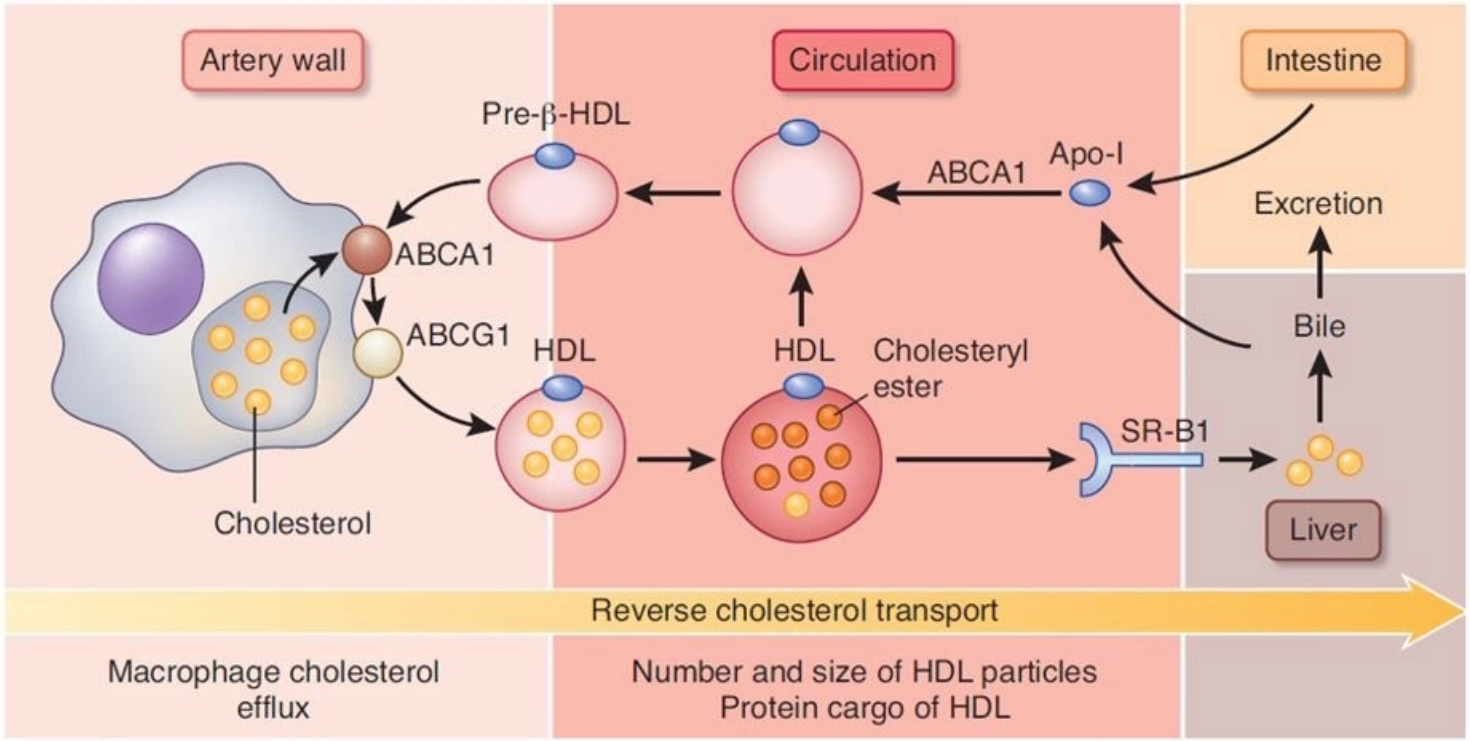

HDL




LDL


LDL



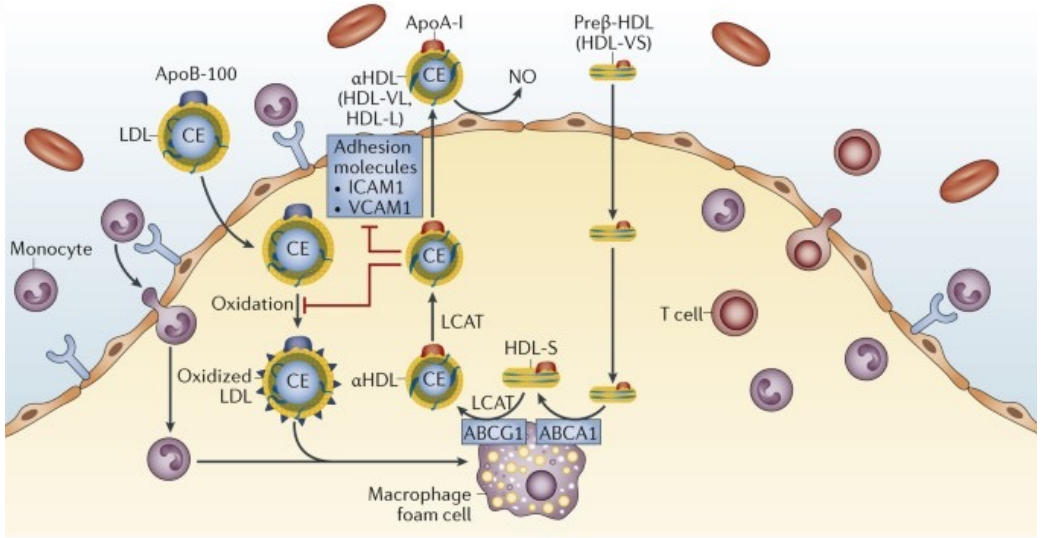


Conventional Wisdom Says HDL is Good Because of Reverse Cholesterol Transport

Challenge:

HDL CEC <u>inversely</u> associates with incident and prevalent CHD in <u>some</u> cohorts, <u>directly</u> in some cohorts.

HDL-CEC <u>directly</u> associates with CAC



Heinecke, Nature, 2012; Linsel Nitscke & Tall Nature Review Drug Discovery 2005

Framingham Heart Study doesn't Capture Race Diversity

Table 1.

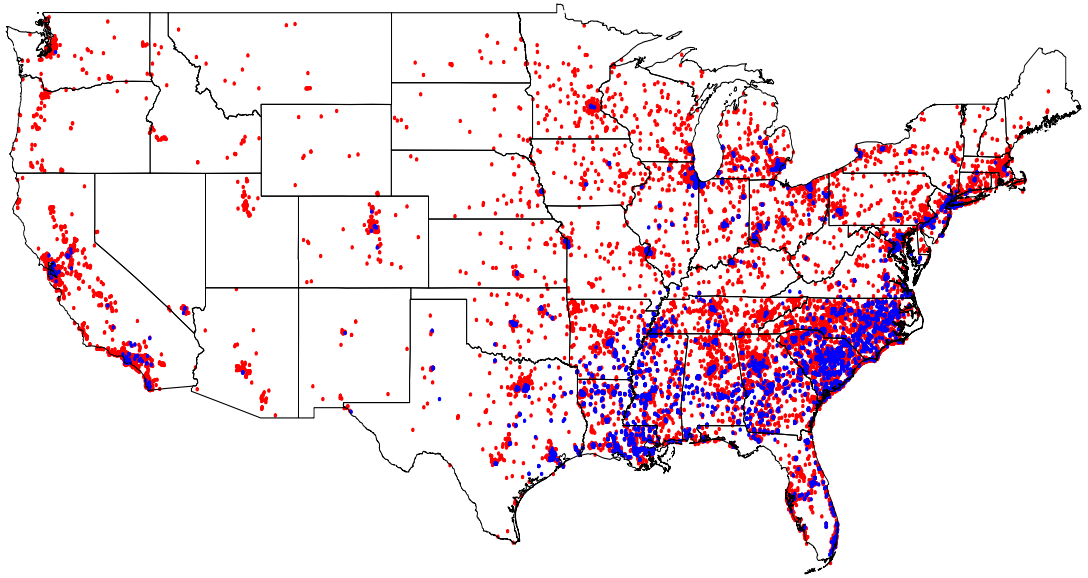
Characteristics of FHS cohorts

Cohort	Total, *n*	DNA, *n*	Ancestry, %					Year recruited	Age, years	Follow-up		
			EA	AA	HA	AsA	Other			Examinations, *n*	Intervals, *n*	Duration, years
Original	5209	971	100	0	0	0	0	1948–53	28–74	32	2	65
Offspring (and spouses)	5124	3930	100	0	0	0	0	1971–75	5–70	9	4–8	43
Third Generation	4095	4077	100	0	0	0	0	2002–05	19–72	2	6	≈ 10
Offspring Spouses	103	101	100	0	0	0	0	2003–05	47–85	2	6	≈ 10
Omni 1 Cohort	507	493	0	28	42	24	6	1994–98	27–78	4	4–8	≈ 15–20
Omni 2 Cohort	410	407	0	28	42	24	6	2003–05	20–80	2	6	≈ 10

AA, African American; AsA, Asian American; EA, European American; HA, Hispanic American.

Table from Benjamin I *et al. Circulation* 2015;**131:**100–112.

Reasons for Geographic and Racial Differences in Stroke (REGARDS)



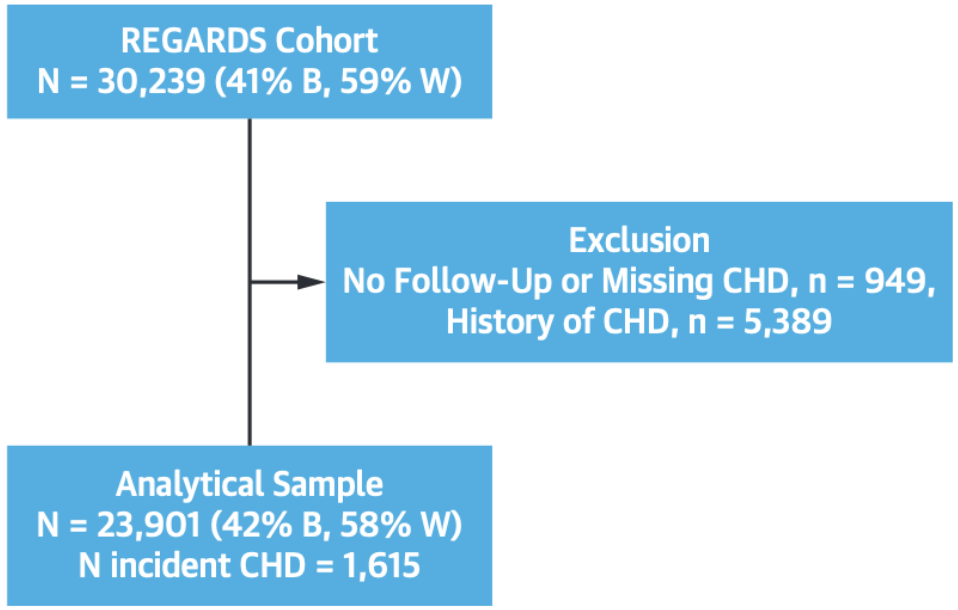
N > 30,000 from the **"Stroke belt"**

- White American (n = 17,428)
- African American (n = 12,128)

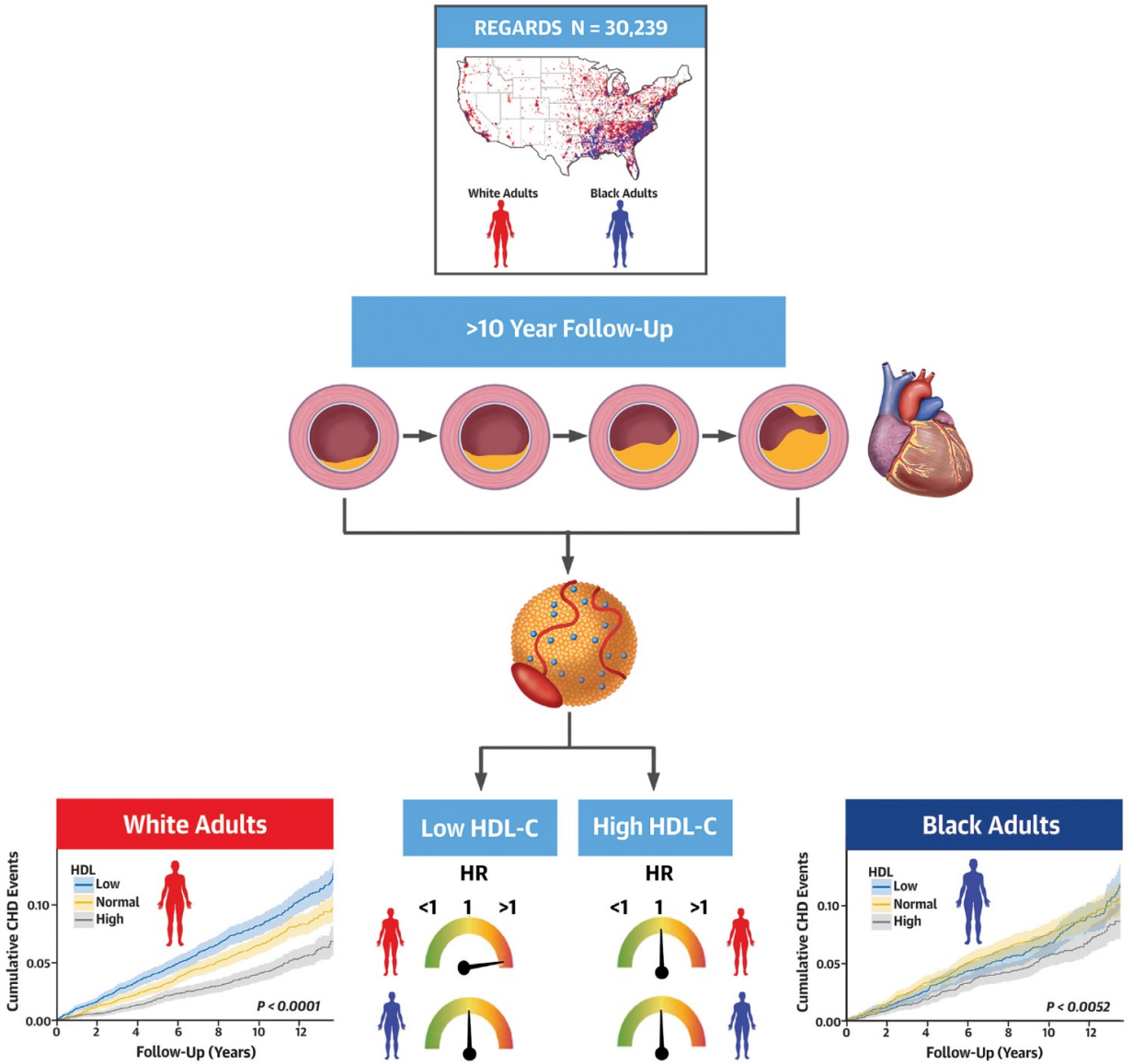
- Longitudinal population-based cohort
  - 30,239
  - age 45 and older
- Random sampling with geographic representation
  - **21% from the buckle of the stroke belt**
  - **35% from the stroke belt**
  - **44% from the rest of the contiguous US**
- 42% African American / 58% white
- 45% male / 55% female

Race-Specific Association of HDL-C with CHD Risk

FIGURE 1 The Flowchart of the Study Cohort



REGARDS (REasons for Geographic and Racial Differences in Stroke) is a national prospective cohort of 30,239 participants composed of community-dwelling Black (B) and White (W) women and men aged ≥45 years, identified via mail and telephone using commercially available lists of U.S. residents, and enrolled from 2003 to 2007. Incident coronary heart disease (CHD) was defined as a definite or probable nonfatal myocardial infarction or CHD death after the baseline in-person visit or on or before December 31, 2017.



Zakai NA, et al. J Am Coll Cardiol. 2022;80(22):2104–2115.

Low high-density lipoprotein cholesterol (HDL-C) levels were detrimental only in White adults; high HDL-C levels were not protective in either race. CHD = coronary heart disease; REGARDS = REasons for Geographic and Racial Differences in Stroke.

Higher HDL-C is associated with reduced CHD risk in Whites but not in Blacks

HDL clinical Categories

- **low (reference):**

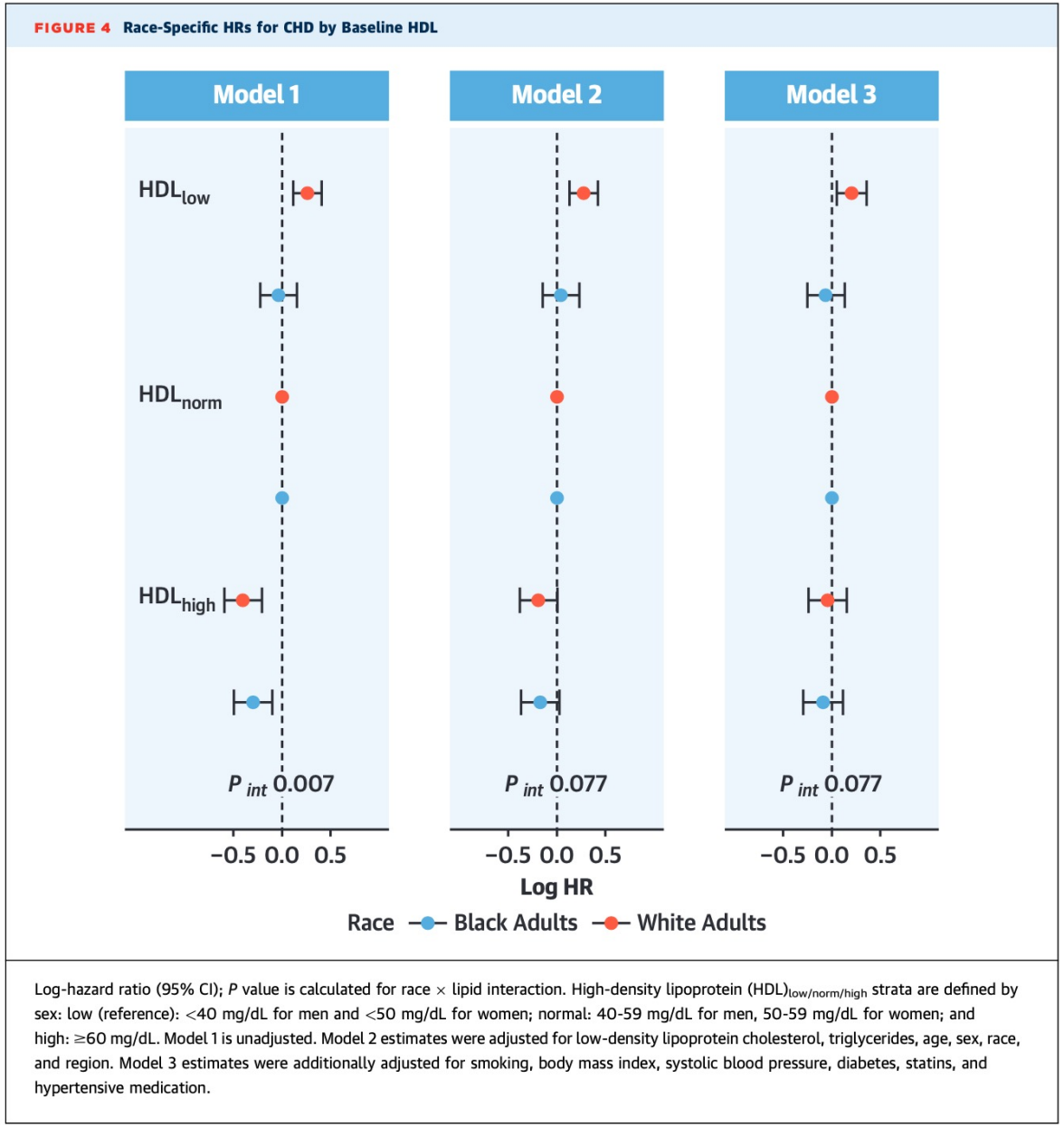
<40mg/dL for males
<50mg/dL for females

- **moderate:**

40-59mg/dL for males,
50-59mg/dL for females

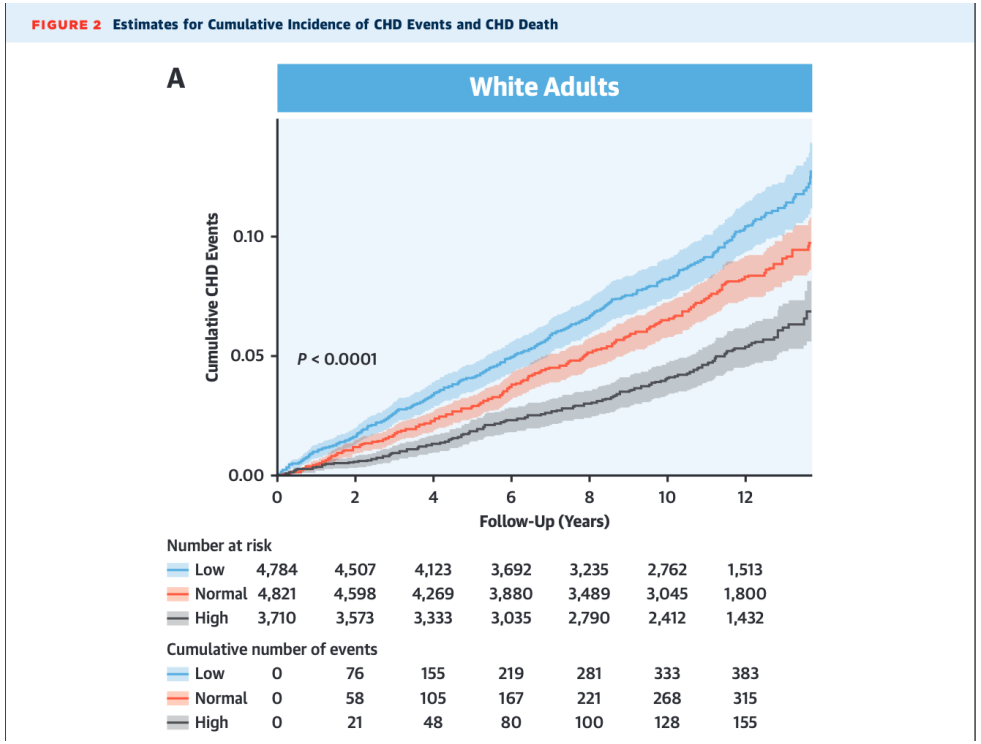
- **high:**

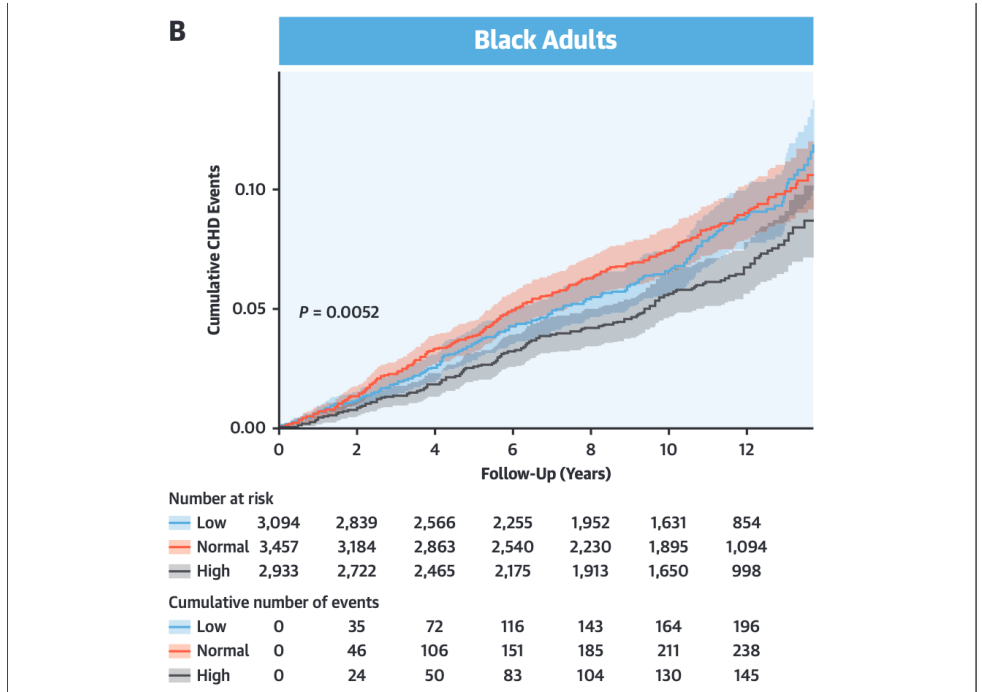
>=60mg/dL.



Zakai et al. JACC 2022

White Adults but not Black Adults Align with Framingham Expectations

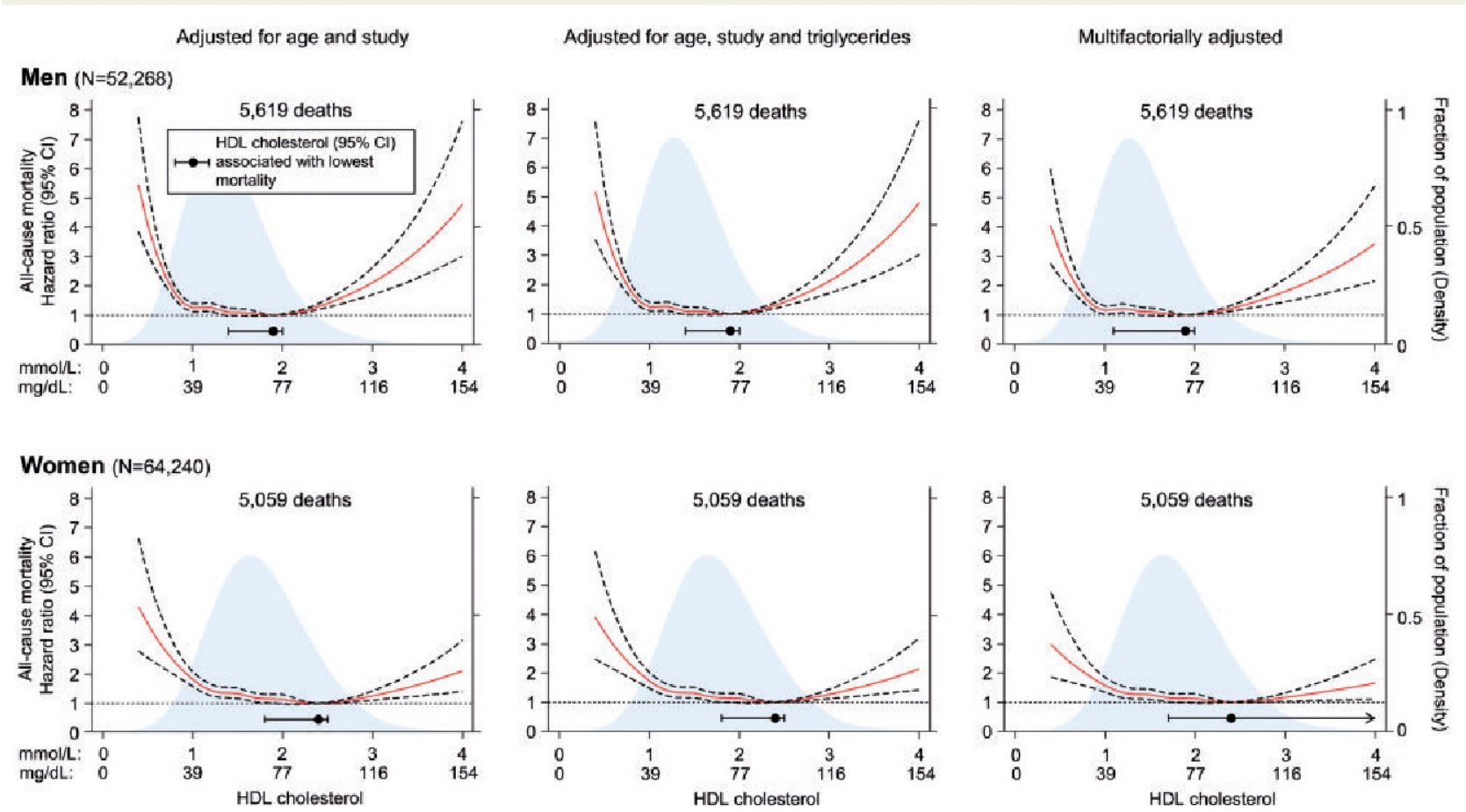




(A) White adults, **(B)** Black adults. Kaplan-Meier estimates were calculated per clinical high-density lipoprotein (HDL) categories. Participants were classified by sex: low (reference): <40 mg/dL for men and <50 mg/dL for women; normal: 40-59 mg/dL for men, 50-59 mg/dL for women; and high: ≥60 mg/dL. *P* values are from log-rank test. CHD = coronary heart disease.

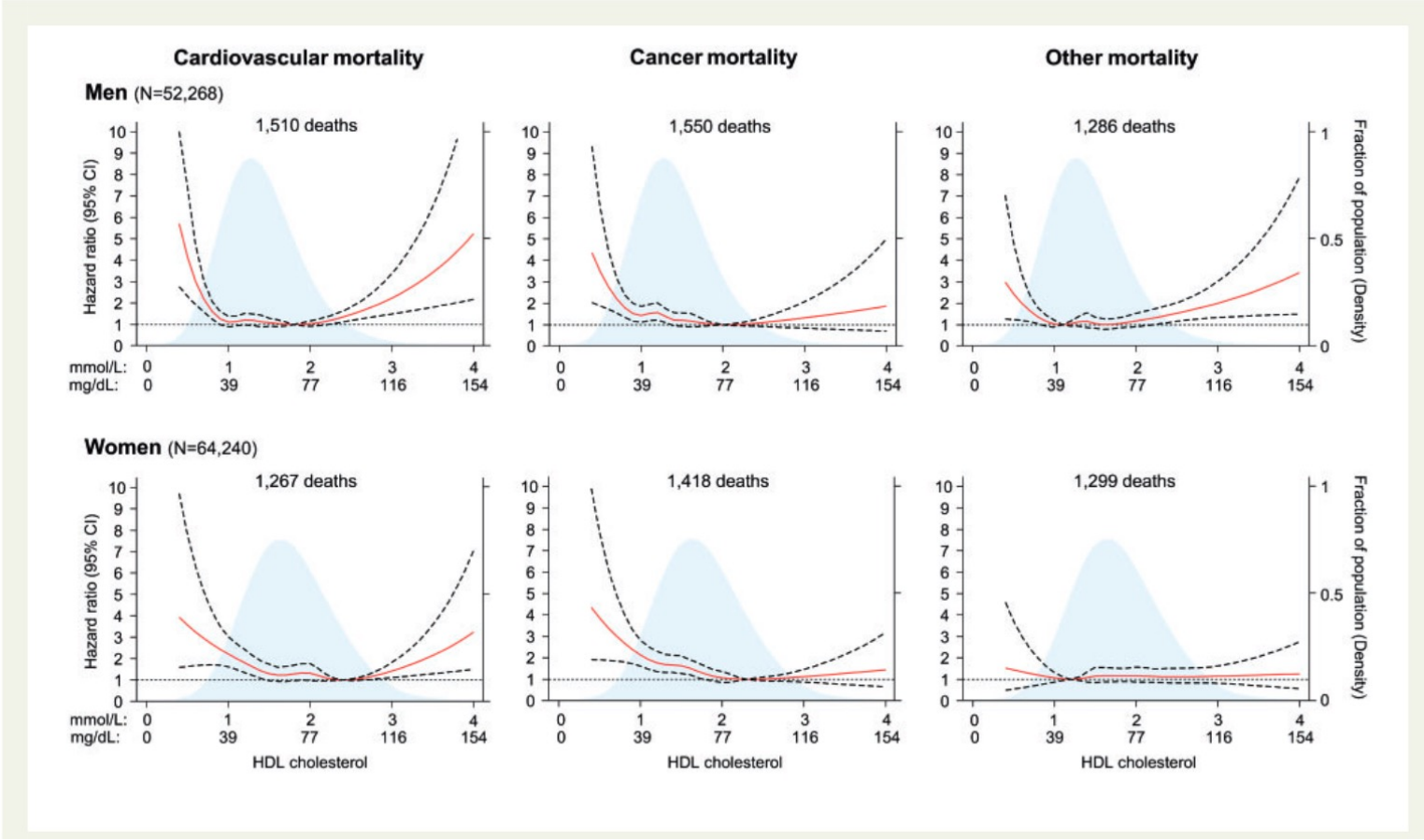
Zakai et al, JACC, 2022

Elevated HDL Cholesterol associated with increased all cause mortality in both men and women



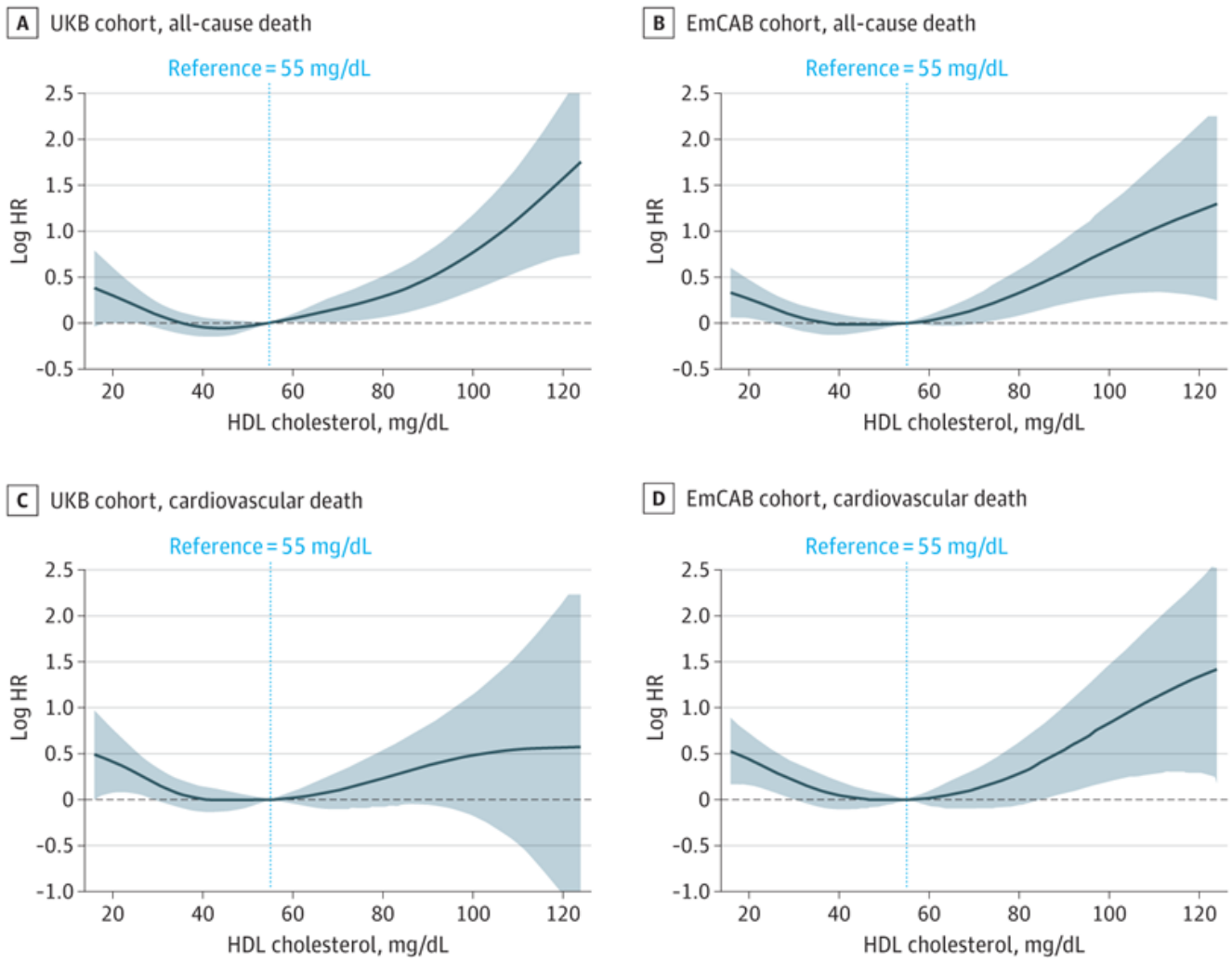
Madsen CM, Varbo A, Nordestgaard BG. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. Eur Heart J. 2017 Aug 21;38(32):2478-2486. doi: 10.1093/eurheartj/ehx163. PMID: 28419274.

HDL-C >80mg/dL Associated with Increased Cardiovascular Mortality



Madsen CM, Varbo A, Nordestgaard BG. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. Eur Heart J. 2017 Aug 21;38(32):2478-2486. doi: 10.1093/eurheartj/ehx163. PMID: 28419274.

High HDL-C associates with adverse cardiovascular outcomes



Liu C et al. *JAMA Cardiol.* 2022

CETP Inhibitors Raise Plasma HDL-C Levels with No Cardiovascular Benefits

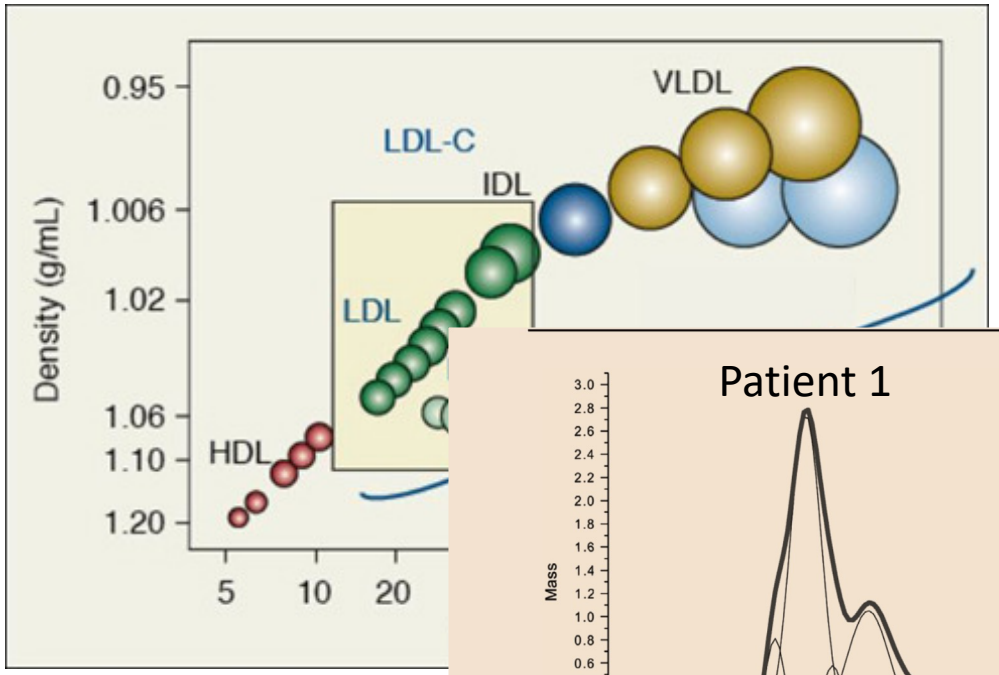
Table. Clinical Outcomes Trials of CETP Inhibitors

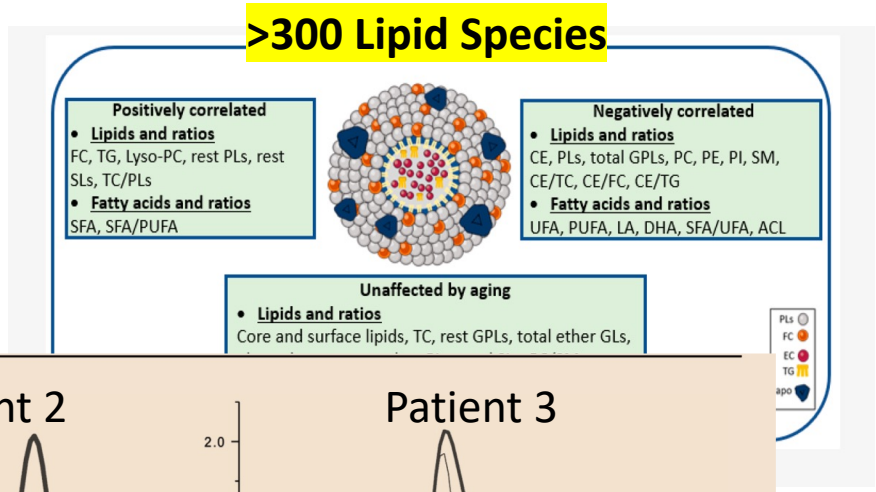
Trial (Drug)	Patients	Lipoprotein Changes	Duration	Outcome	Comments
ILLUMINATE (torcetrapib)	15067 hi CV risk	HDL-C↑ 72%LDL-C ↓*	1–2 y	↑CV events↑Death↑SBP (5 mm)	Electrolyte disturbances, hyeraldosteronism identified as off target effects*LDL measured indirectly
dal-OUTCOMES (dalcetrapib)	15871 post ACS	HDL-C↑ ≈30%LDL-C→	31 mo	CV events→↑SBP (0.6 mm)	Trial stopped early for futility. Possible benefit in a genetic subgroup
ACCELERATE (evacetrapib)	12092 hi risk vascular disease	HDL-C↑ 133%LDL-C↓*	26 mo	CV events→↑SBP (1.2 mm)	Trial stopped early for futility↓Deaths (not prespecified)*LDL measured indirectly
REVEAL (anacetrapib)	30449 hi risk vascular disease	HDL-C↑ 104%LDL-C↓ 17%	4.1 y	↓Coronary events↑SBP (0.7 mm)	Trial went to planned completion↓new onset diabetes mellitus

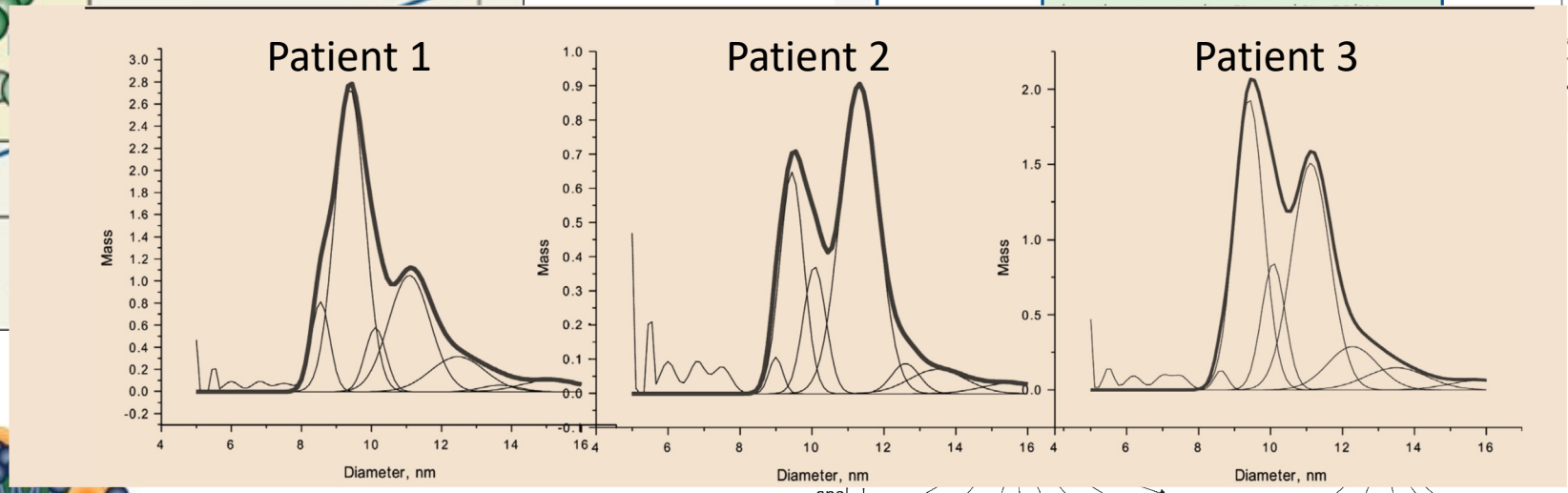
ACCELERATE indicates Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition with Evacetrapib in Patients with a High Risk for Vascular Outcomes; ACS, acute coronary syndromes; CETP, cholesteryl ester transfer protein; CV, cardiovascular; HDL-C, high-density lipoprotein cholesterol; ILLUMINATE, The Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events Outcomes; LDL-C, low-density lipoprotein cholesterol; REVEAL, Randomized Evaluation of the Effects of Anacetrapib through Lipid modification; and SBP, systolic blood pressure.

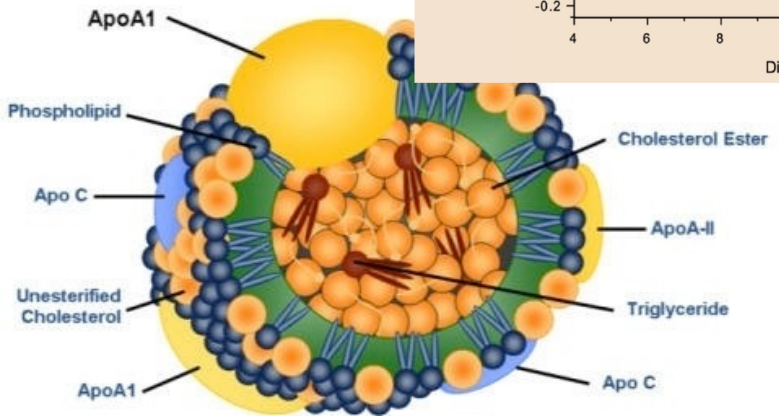
Tall and Rader, Circ Res, 2017

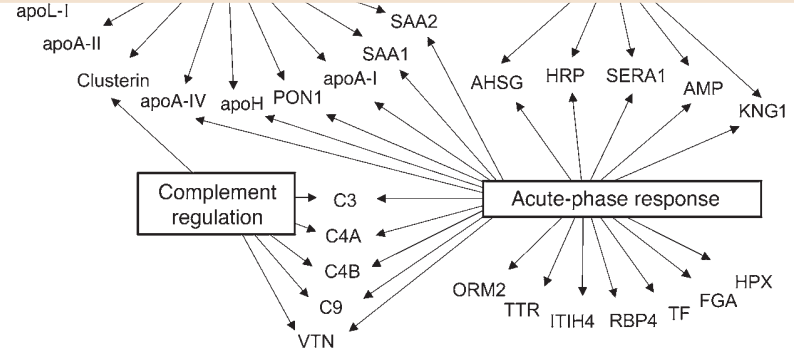
HDL is a Heterogenous Particle



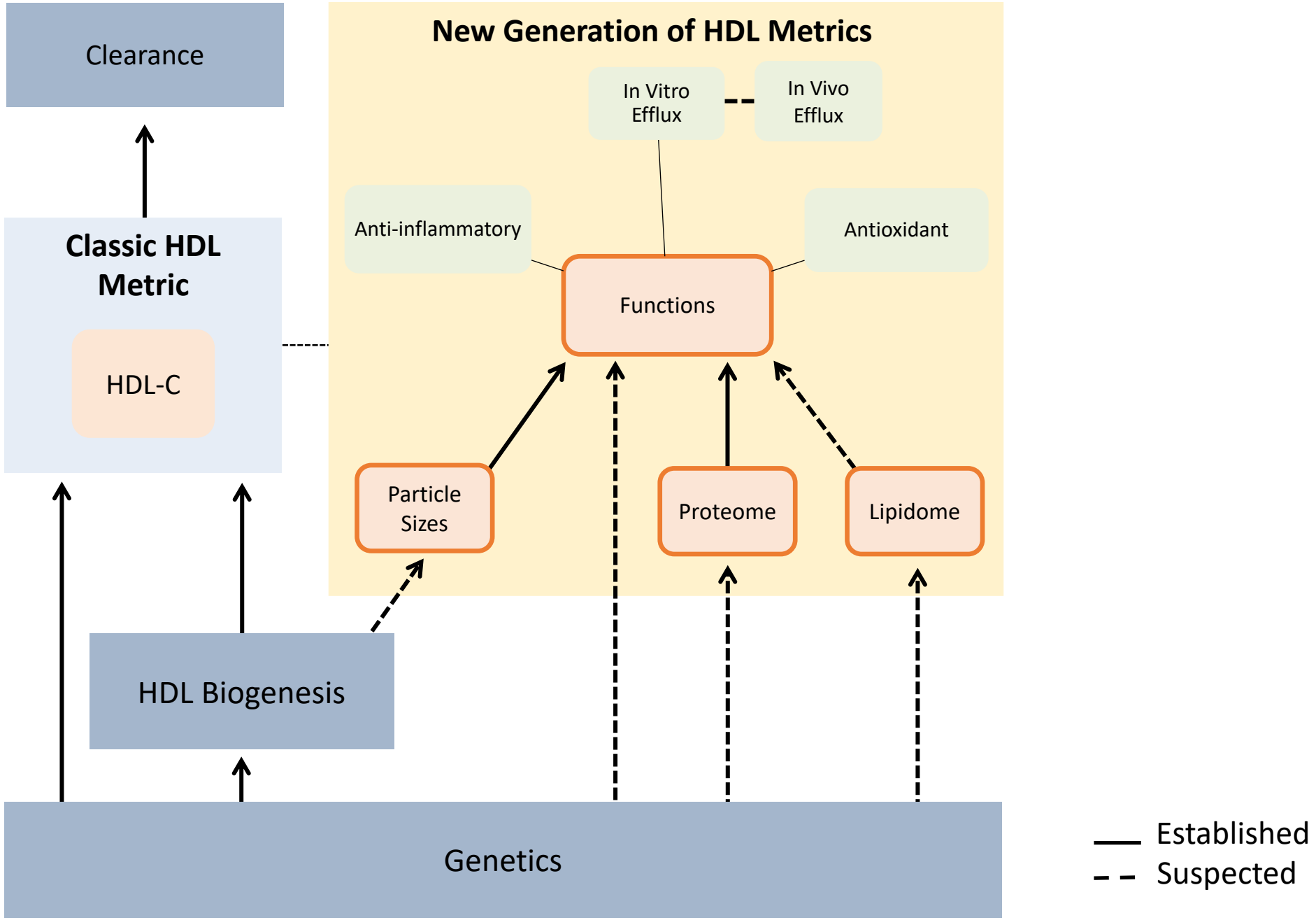








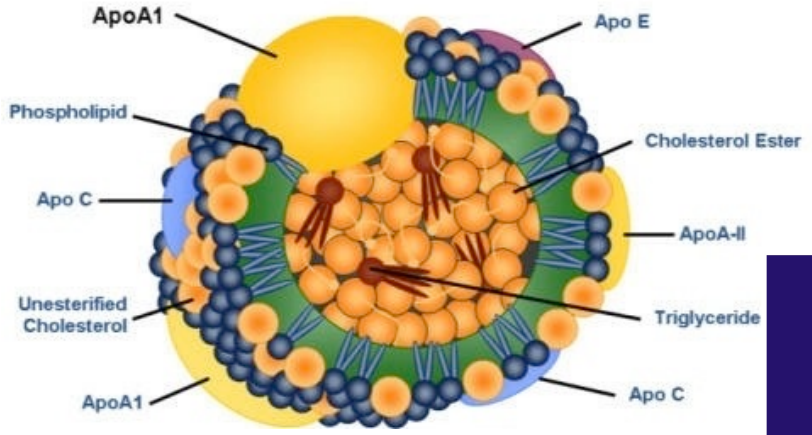
Vaisar et al. JCI, 2007



Fazio and Pamir, Circ Res, 2016

Dysfunctional HDL is Linked to Disease

Functional HDL Particle



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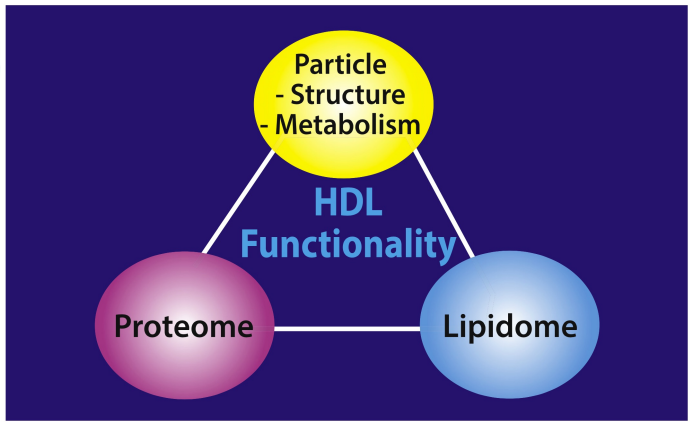


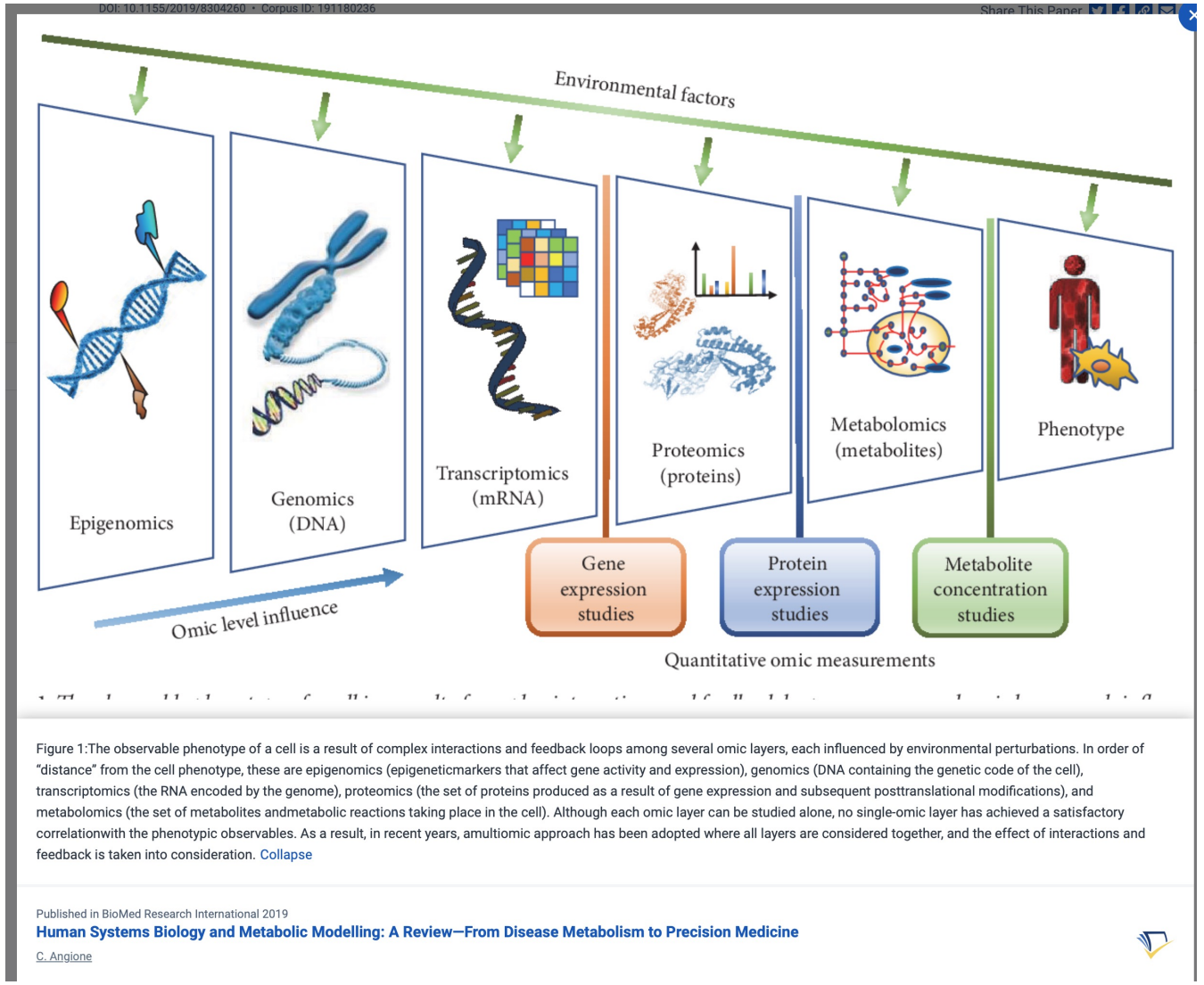
Dysfunctional HDL Particle

Adapted from Ben-Aisha, IJMS, 2020

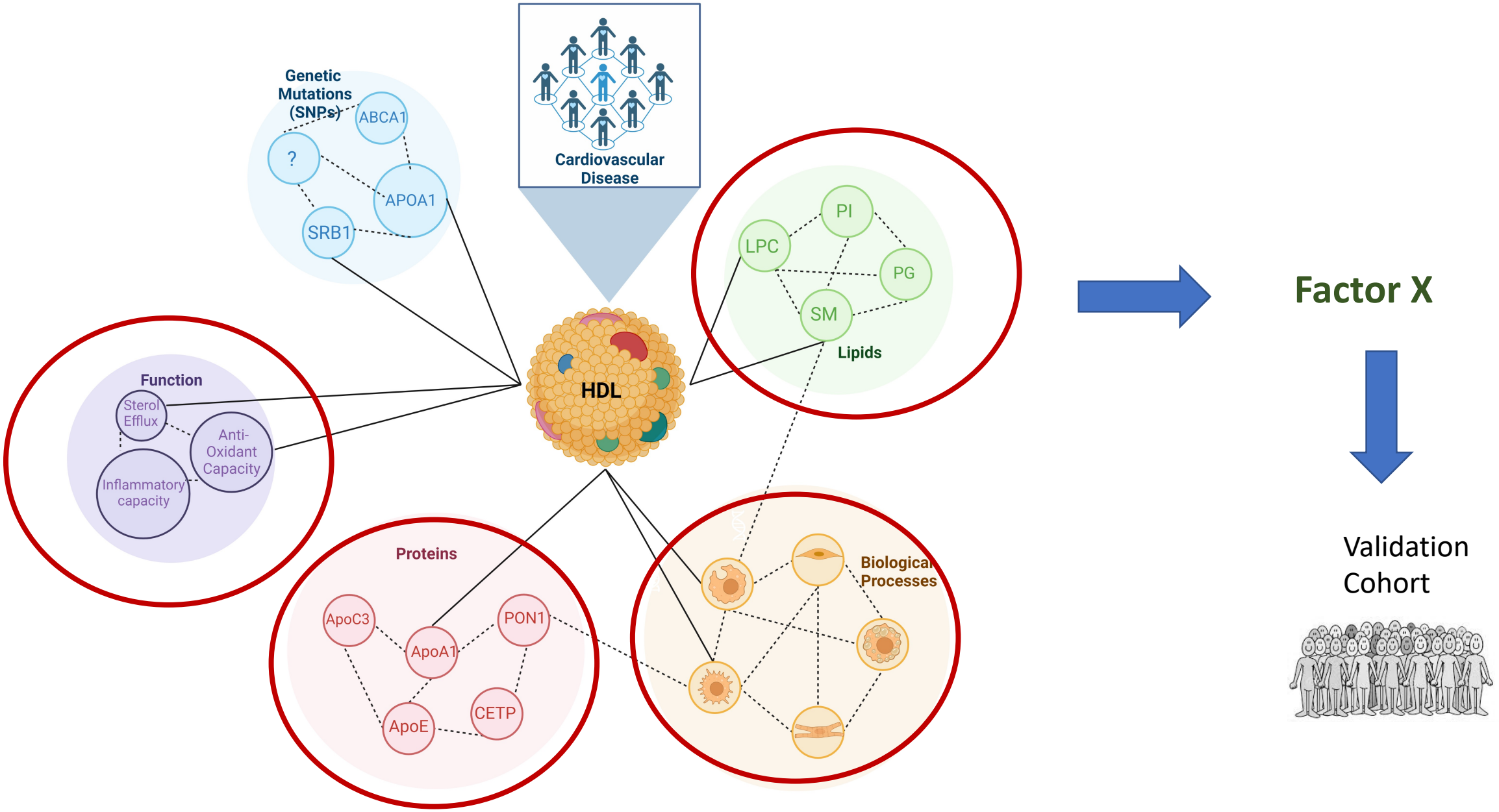
Systems Biology as Defined by NIH

Systems biology is an approach in biomedical research to understand the larger picture—be it at the level of the organism, tissue, or cell—by putting its pieces together. It's in stark contrast to decades of reductionist biology, which involves taking the pieces apart.

May 2, 2022



Clinical Systems Biology Approach



Center For Preventive Cardiology Registry and Biorepository

Is HDL detrimental in patients that have high HDL and high CAC?

Hypothesis: HDL lipid and protein composition makes HDL dysfunctional

Study HDL composition & function in patients with a defined CVD marker, coronary artery calcification (CAC) score

Composition

- Proteomics
- Lipidomics

Function

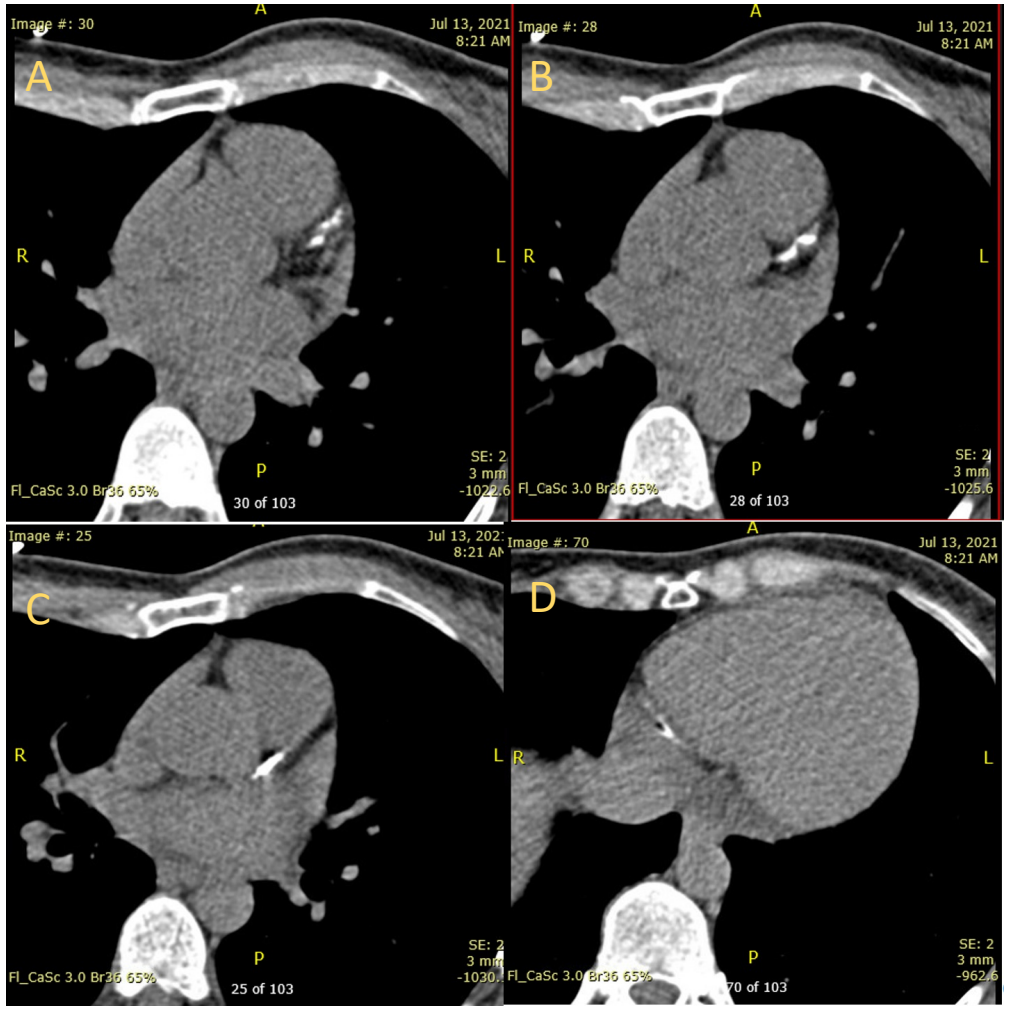
- Cholesterol efflux capacity
- Anti-inflammatory capacity

Project Goal

Study HDL composition & function in patients with a defined CVD marker, coronary artery calcification (CAC) score

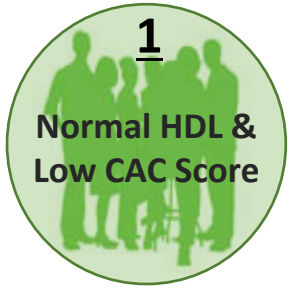
Composition	Function
➢ Proteomics	➢ Cholesterol efflux capacity
➢ Lipidomics	➢ Anti-inflammatory capacity

Coronary Artery Calcification Visualization

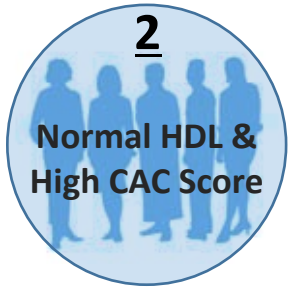


Computed Tomography Scan: The calcification of the left anterior descending artery (A-C) and the right coronary artery D) .

Cohort Characteristics



HDL-C <80 mg/dL
CAC score <100



HDL-C <80 mg/dL
CAC score >100

HDL-C >80 mg/dL
CAC score <100

HDL-C >80 mg/dL
CAC score >100

Cohort Characteristics	**Group 1** Normal HDL & Low CAC	**Group 2** Normal HDL & High CAC	**Group 3** High HDL & Low CAC	**Group 4** High HDL & High CAC
Total Patients	36	41	15	10
Average BMI (± SD)	29.5 ± 5.3	29.3 ± 5.4	25.7 ± 2.5	25.0 ± 4.2
Average Age (± SD)	59.8 ± 12.0	64.1 ± 9.1	65.9 ± 9.8	66.8 ± 11.5
Gender (% Female)	44.4%	46.3%	73.3%	75.0%
Average CAC Score (± SD)	**18.7 ± 26.3**	**582.1 ± 707.8**	**15.1 ± 23.9**	**376.9 ± 190.2**
Median CAC Score [IQR]	**2.3 [0-31.9]**	**386.8 [179-581]**	**1.0 [0-19.9]**	**440.7 [170.5-523.6]**
Average HDL-C (mg/dL ± SD)	**50.9 ± 7.4**	**52.3 ± 10.3**	**94.3 ± 14.8**	**96.5 ± 15.4**
Average LDL-C (mg/dL ± SD)	158.9 ± 88.0	107.0 ± 56.7	125.7 ± 68.9	142.8 ± 118.5
Average VLDL-C (mg/dL ± SD)	50.4 ± 66.7	32.9 ± 16.1	21.9 ± 11.6	16.7 ± 7.8
Average Lp(a) (mg/dL ± SD)	46.4 ± 50.7	51.3 ± 60.5	59.5 ± 81.2	48.2 ± 46.5
Average TC (mg/dL ± SD)	257.0 ± 119.2	196.0 ± 68.6	239.1 ± 66.0	255.7 ± 120.1
Average Triglycerides (mg/dL ± SD)	240.2 ± 232.0	177.7 ± 106.9	95.3 ± 51.7	82.1 ± 37.7

Proteome remodeling in patients with high HDL and high CAC scores

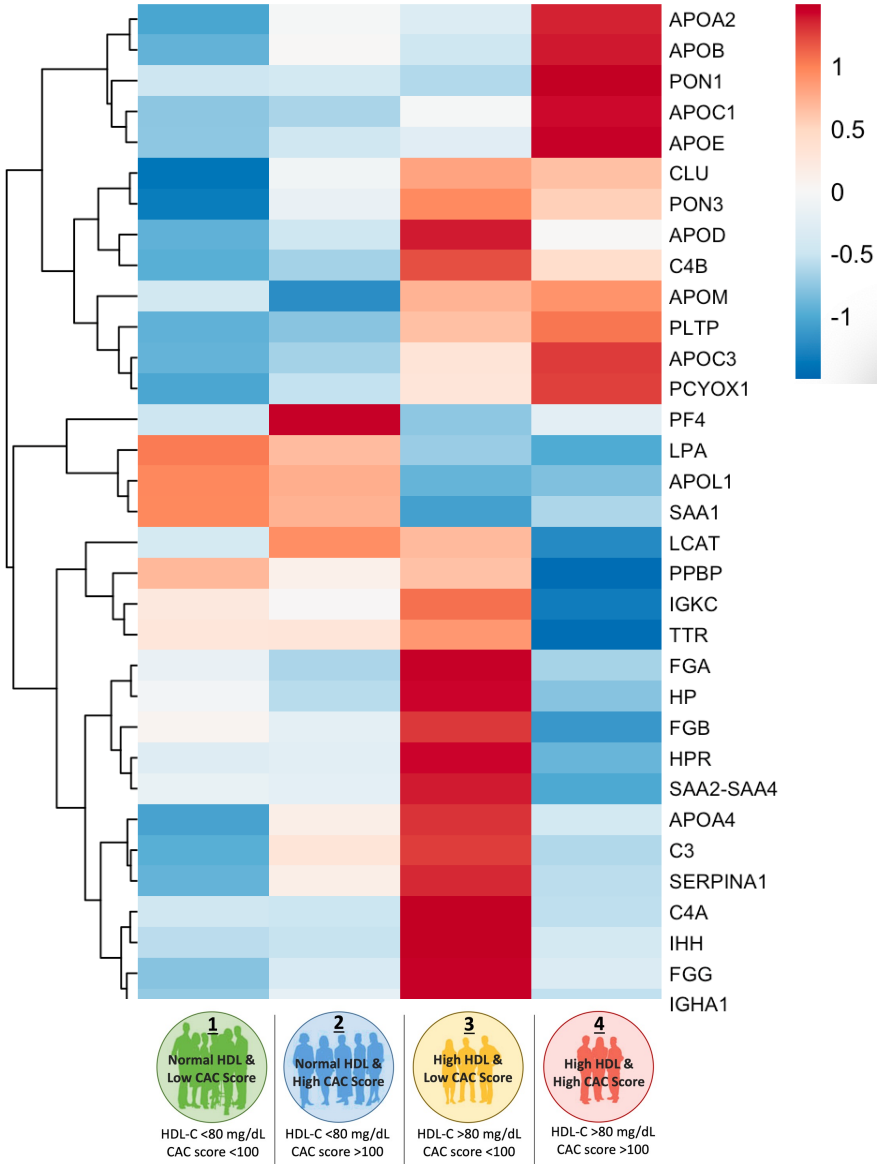
Proteins Detected

297 proteins detected in at least *one* sample
(out of 100 total)

36 proteins detected in >75% of samples in at least *one* group

NHDL = Normal HDL
HHDL = High HDL (>80 mg/dL)
LCAC = Low CAC score
HCAC = High CAC score (>100)

Proteome

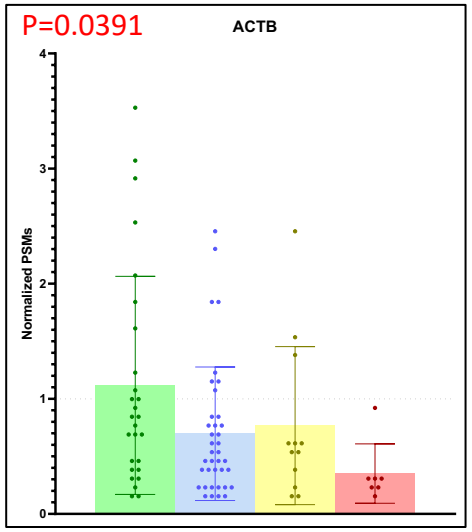


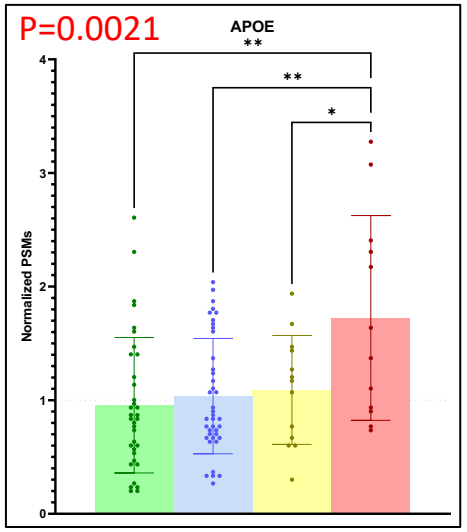
Bergstrom, Hay, Mueller, Pacheco, and Rosario, in preparation

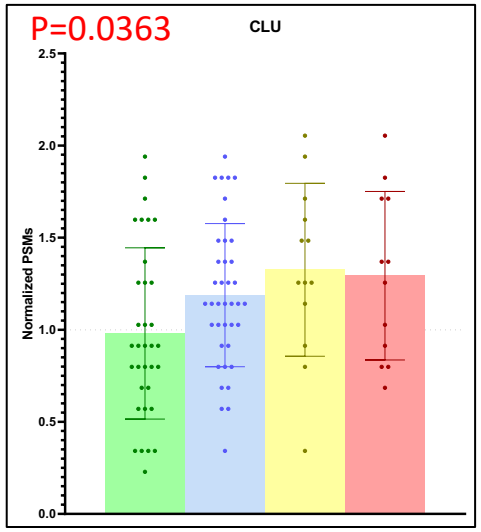
Clusterin and APOE are significantly more abundant on HDL from patients with high CAC scores

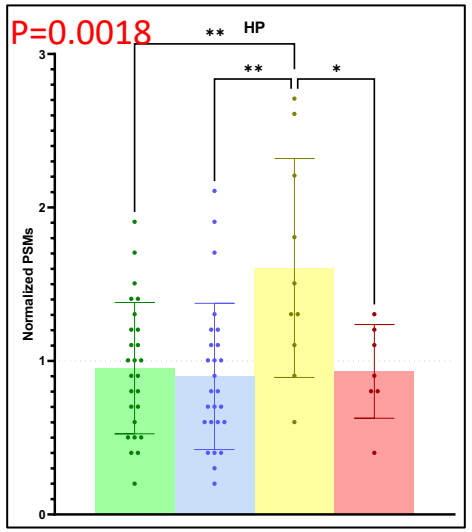
Protein	Sig	One-way ANOVA
ACTB	*	P=0.0391
APOA1	*	P=0.0105
APOE	**	P=0.0021
CLU	*	P=0.0363
HP	**	P=0.0018
HPR	*	P=0.0140
PPBP	ns	P=0.06554

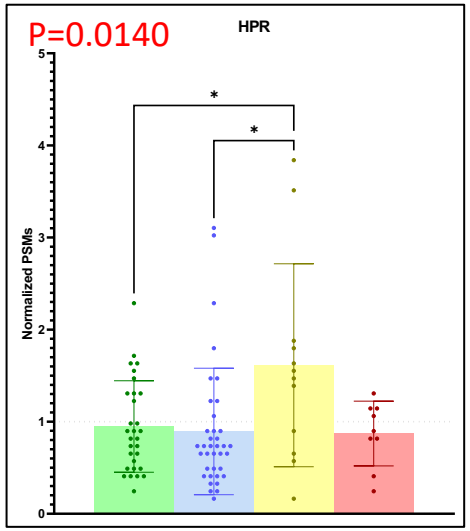
- NHDL / LCAC
- NHDL / HCAC
- HHDL / LCAC
- HHDL / HCAC









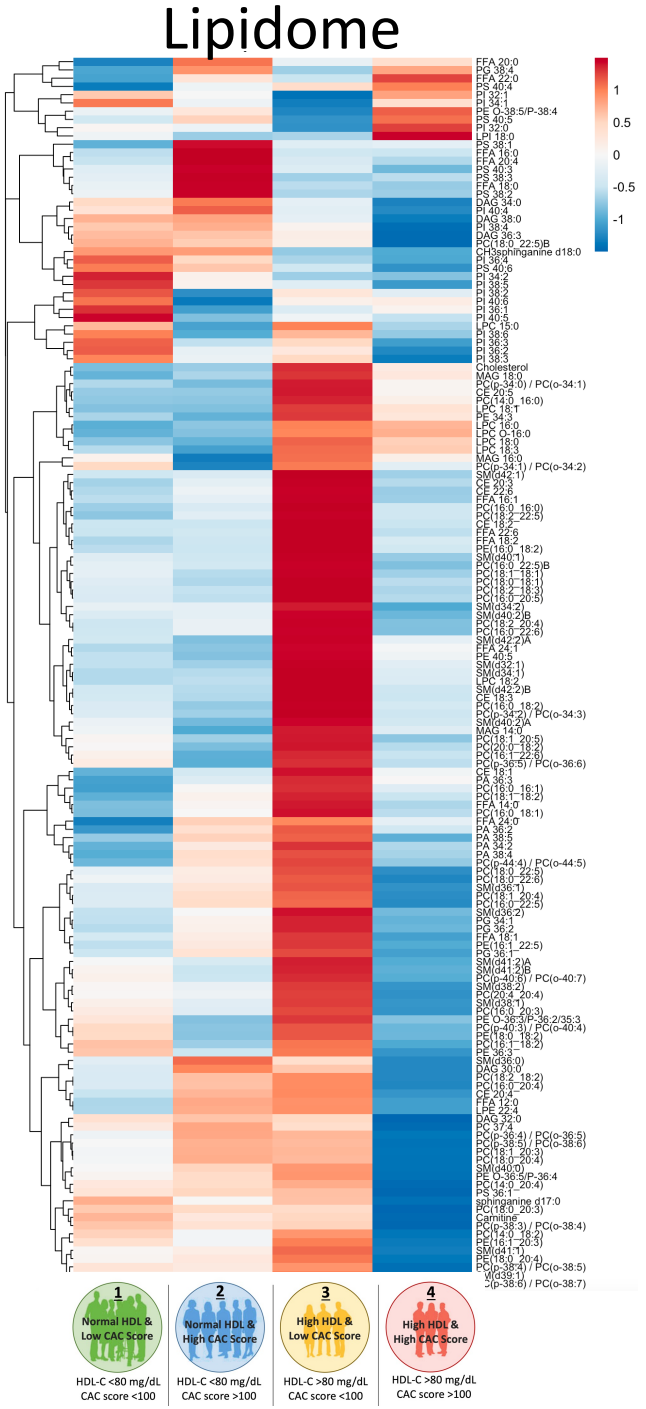


Bergstrom, Hay, Mueller, Pacheco, and Rosario, in preparation

Lipidome remodeling in patients with high HDL and high CAC scores

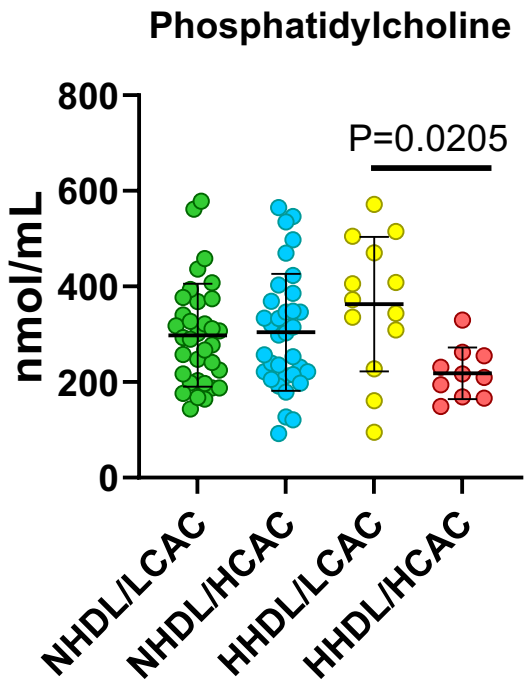
149 Lipid Species detected in 13 different lipid classes

1. 19 SM, Sphingomyelin
2. 8 CE/C, Cholesterol Ester
3. 5 DAG, Diacylglycerol
4. 13 FFA, Free Fatty Acids
5. 7 LPC, Lysophosphatidylcholine
6. 1 LPE, Lysophosphatidylethanolamine
7. 3 MAG, Monoacylglycerol
8. 5 PA, Phosphatidic Acid
9. 45 PC, Phosphatidylcholine
10. 11 PE, Phosphatidylethanolamine
11. 4 PG, Phosphatidylglycerol
12. 17 PI/LPI, Phosphatidylinositol
13. 8 PS, Phosphatidylserine

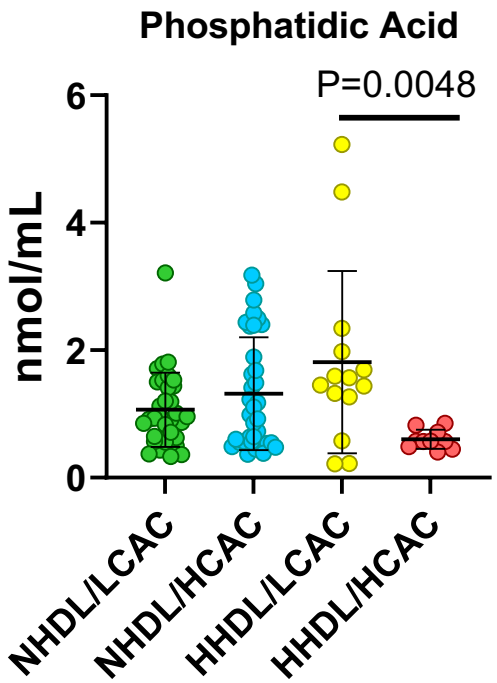


Lipid species, PC, PA, and SM Were Reduced in high HDL/high CAC patients

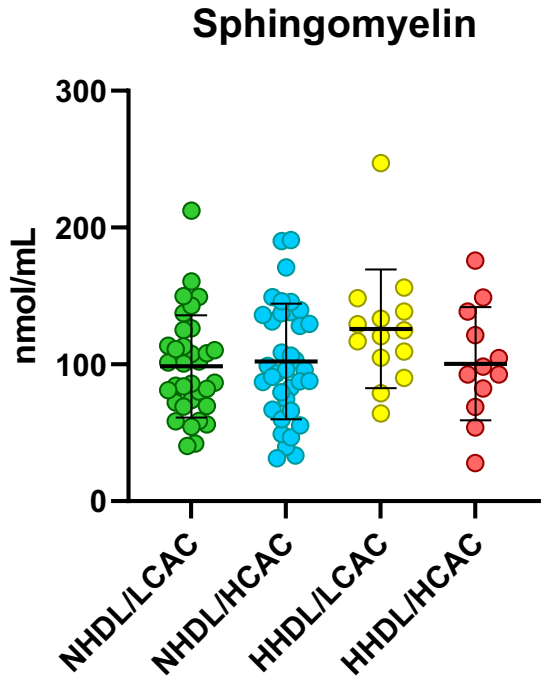
[45 PC species totaled]

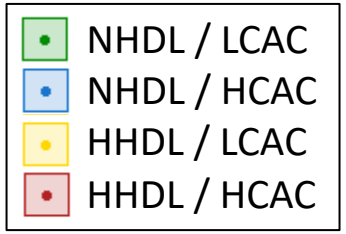


[5 PA species totaled]



[19 SM species totaled]





Anti-Inflammatory Capacity of HDL is Measured with a Cell-based Assay

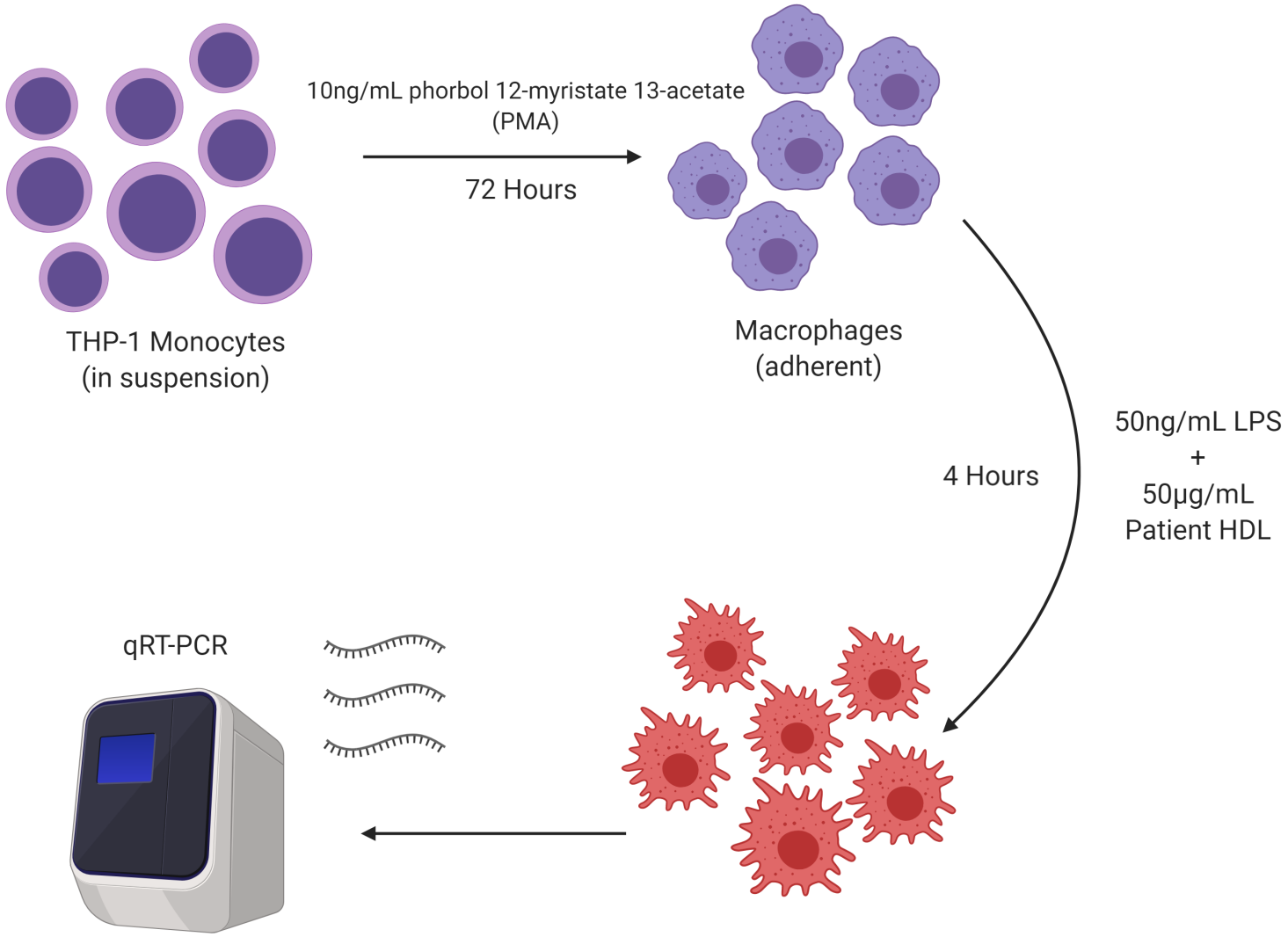
LPS Treatment	HDL Treatment
▲TNF-α	▼TNF-α
▲IL-1β	▼IL-1β
▲IL-6	▼IL-6
▲IL-10	▼IL-10

Gene Targets

IL10

IL6

IL1B

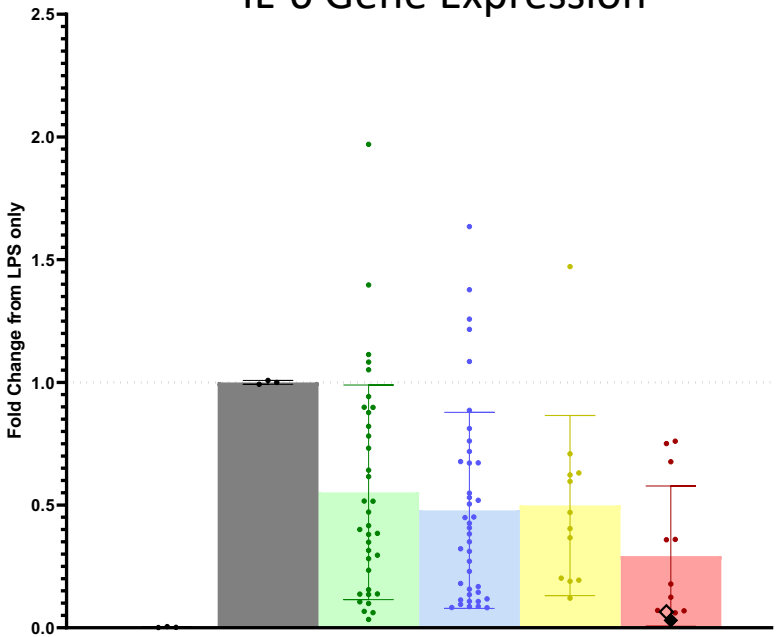


The Anti-inflammatory Capacity of HDL is Enhanced in High CAC Patients

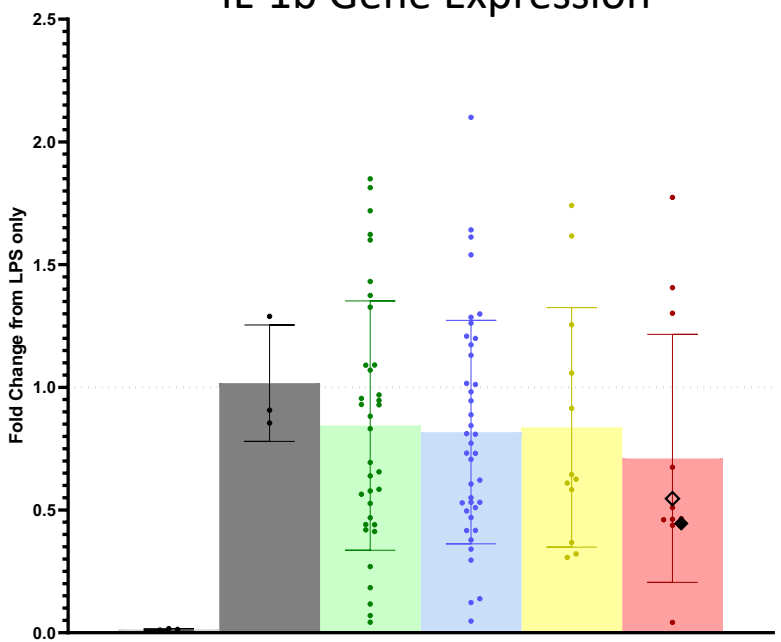
- Vehicle
- LPS only
- NHDL / LCAC
- NHDL / HCAC
- HHDL / LCAC
- HHDL / HCAC

NHDL = Normal HDL (<80 mg/dL)
HHDL = High HDL (>80 mg/dL)
LCAC = Low CAC score (<100)
HCAC = High CAC score (>100)

IL-6 Gene Expression



IL-1b Gene Expression

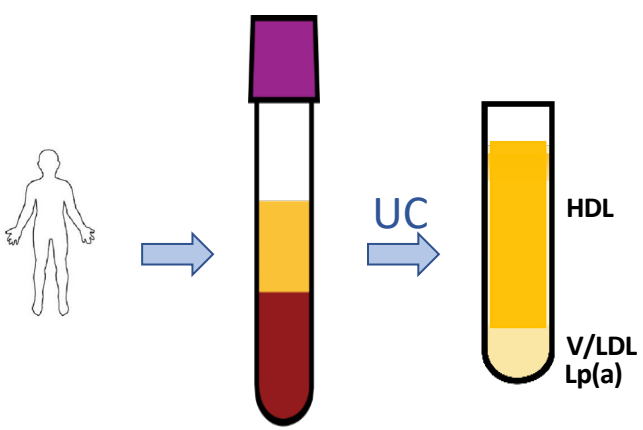


Corresponding labs

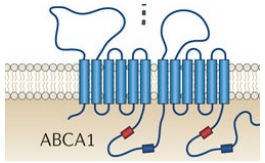
	Sample	CAC Score	HDL-C	Lp(a)	LDL-C	VLDL-C	TC	Triglycerides
◆	2019	566	96	12	141	9	246	45
◇	2021	566	135	12	140	10	285	52

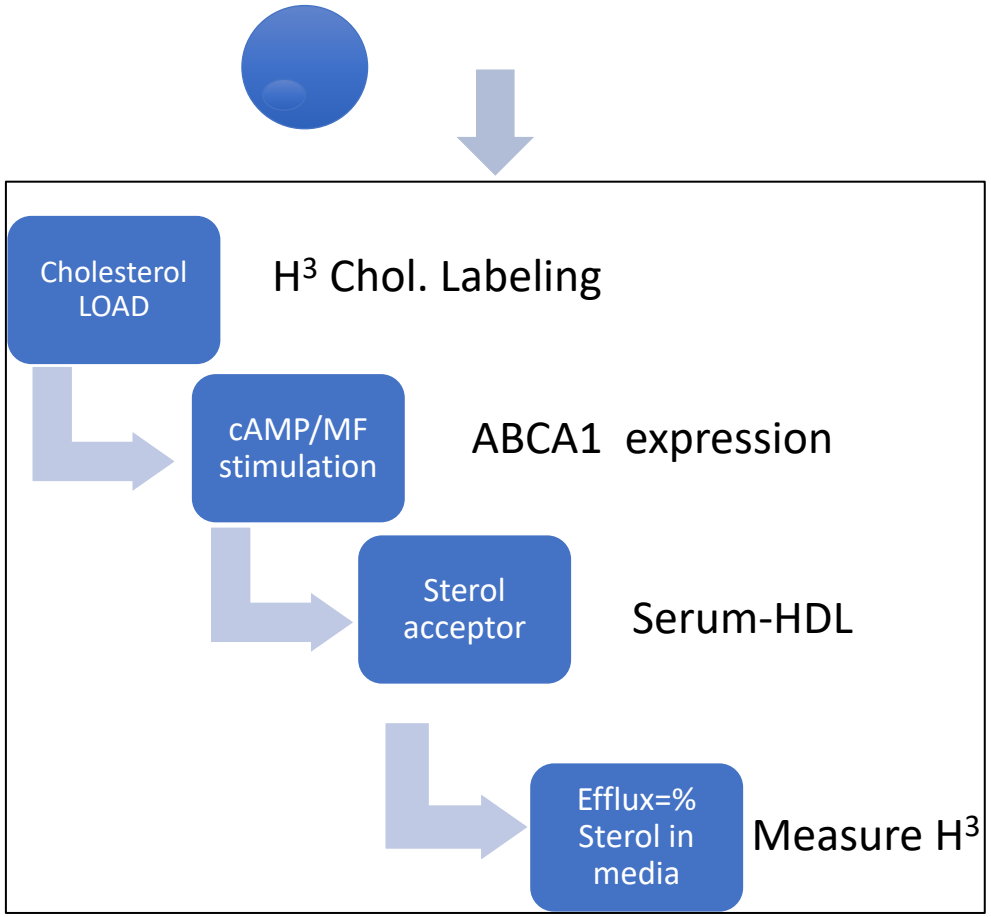
Bergstrom, Hay, Mueller, Pacheco, and Rosario, in preparat

Methods to Measure Sterol Efflux Capacity



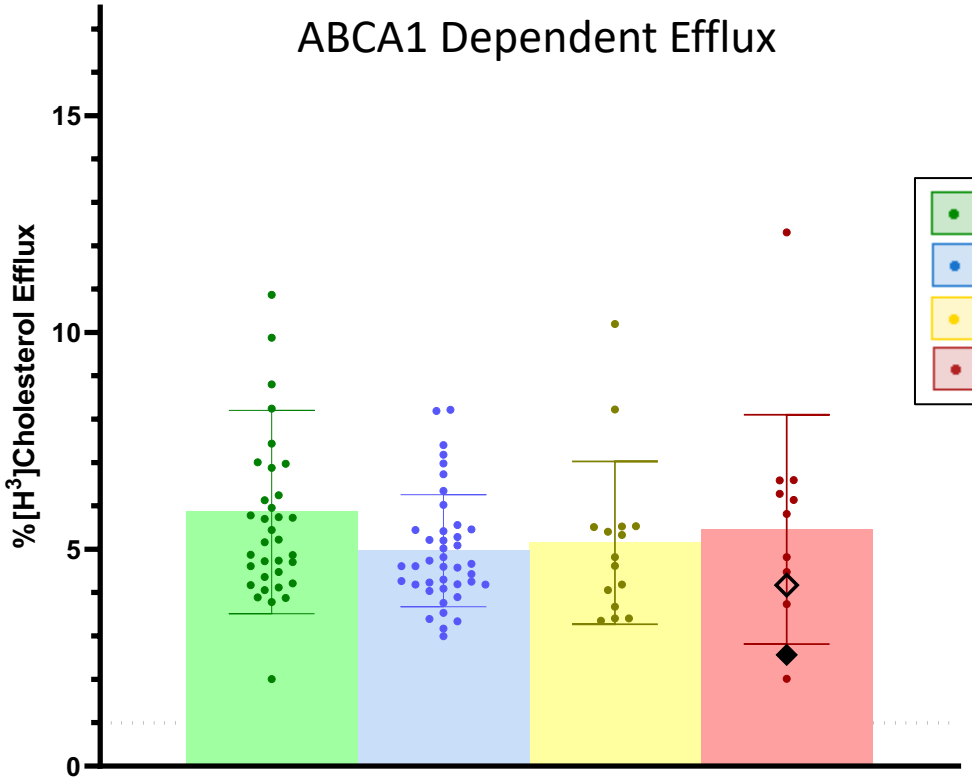
Fibroblasts





ABCA1 Efflux= +ABCA1 - (-)ABCA1

Sterol Efflux Capacity of HDL did not track with CAC scores



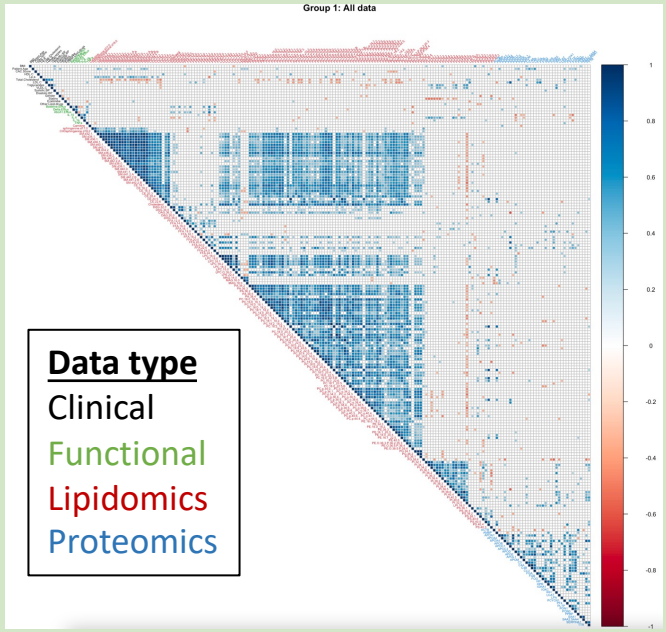
NHDL = Normal HDL (<80 mg/dL)
HHDL = High HDL (>80 mg/dL)
LCAC = Low CAC score (<100)
HCAC = High CAC score (>100)

Corresponding labs

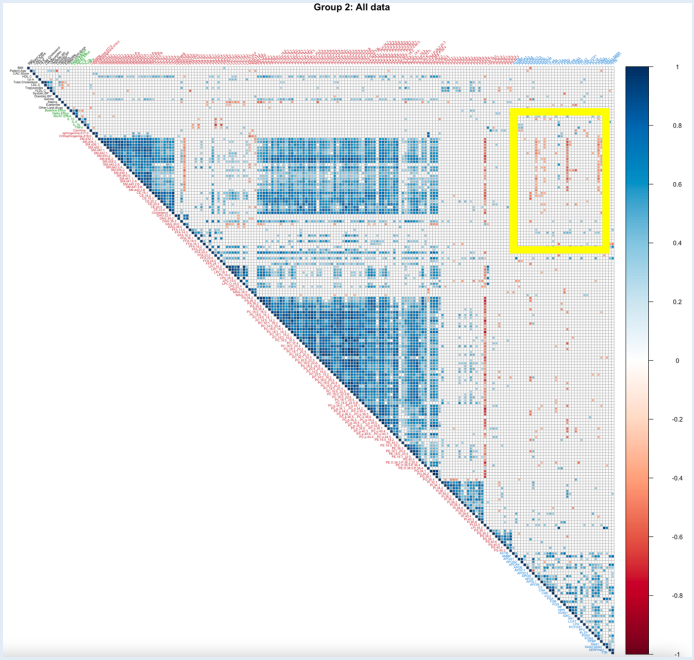
Sample	CAC Score	HDL-C	Lp(a)	LDL-C	VLDL-C	TC	Triglycerides
◆ 2019	566	96	12	141	9	246	45
◇ 2021	566	135	12	43	10	285	52

Bergstrom, Hay, Mueller, Pacheco, and Rosario, in preparation

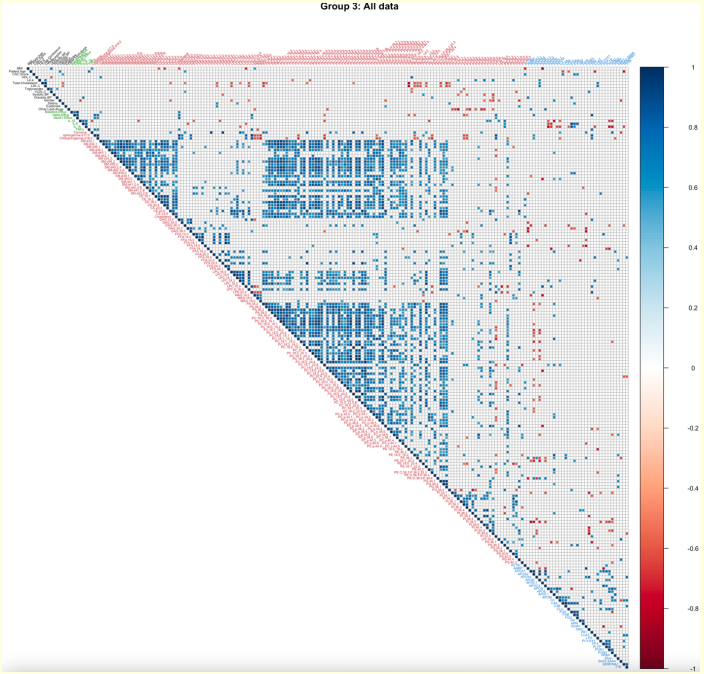
1
Normal HDL &
Low CAC Score
n=36



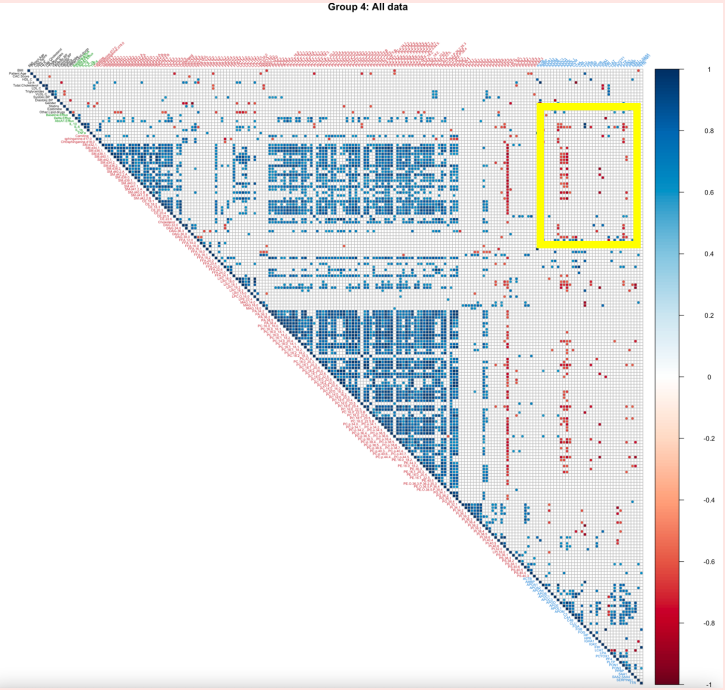
2
Normal HDL &
High CAC Score
n=41



3
High HDL &
Low CAC Score
n=15



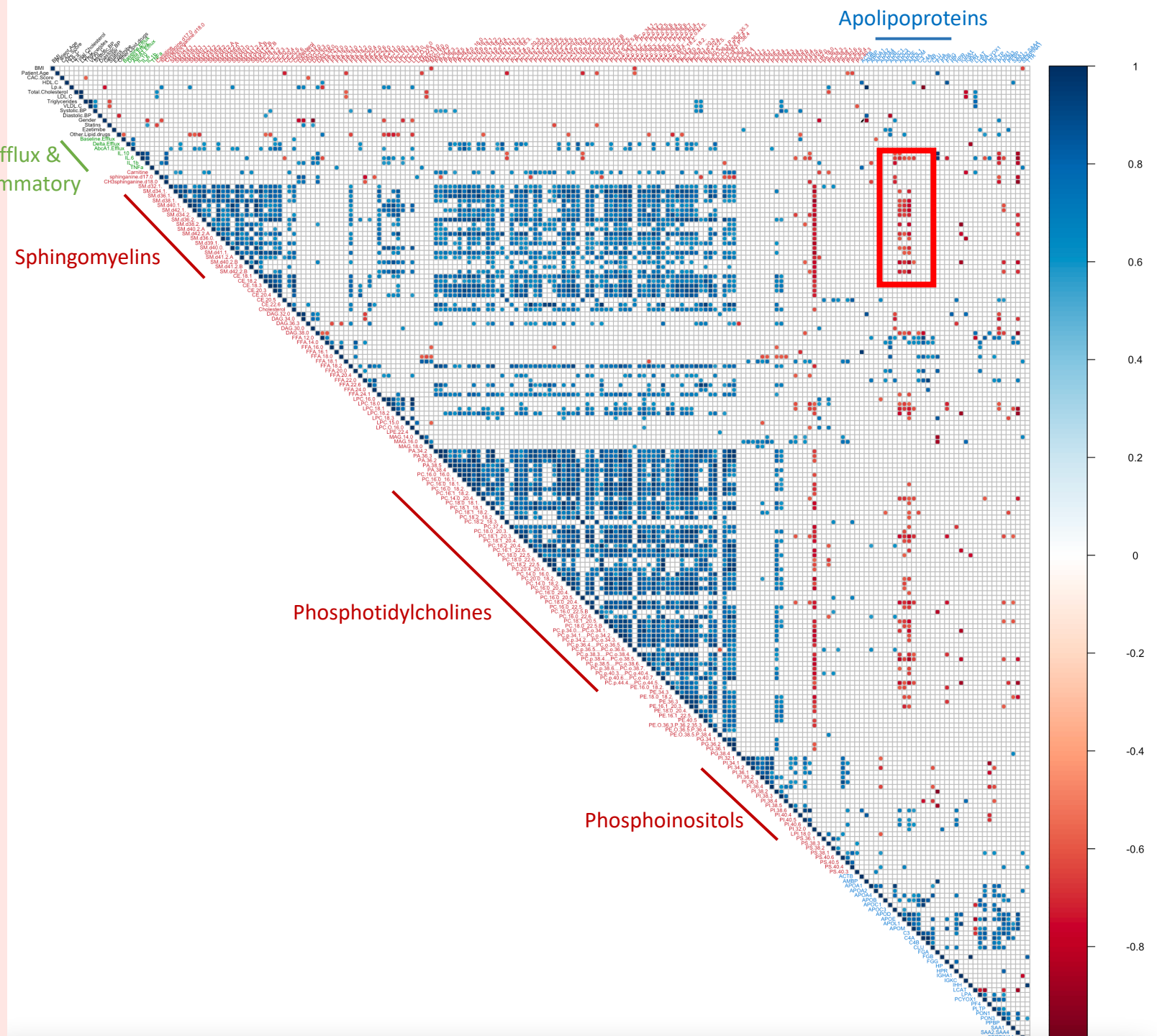
4
High HDL &
High CAC Score
n=10



Bergstrom, Hay, Mueller, Pacheco, and Rosario, in preparation

4
High HDL &
High CAC Score
n=10

Data type
Clinical
Functional
Lipidomics
Proteomics



Bergstrom, Hay, Mueller, Pacheco, and Rosario, in preparation

APOE vs Cholesterol Efflux

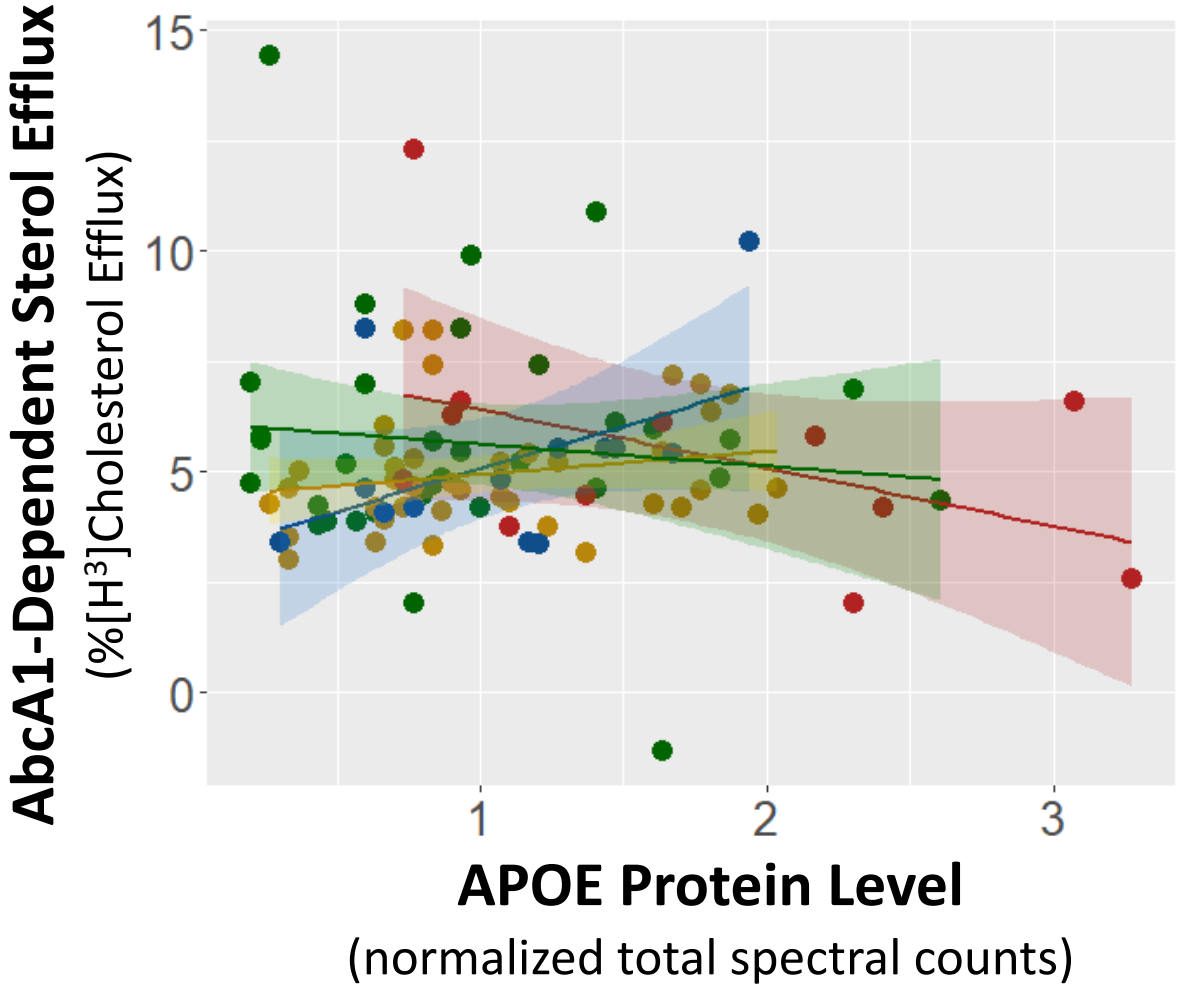
NHDL / LCAC
NHDL / HCAC
HHDL / LCAC
HHDL / HCAC

1
Normal HDL & Low CAC Score
n=36

2
Normal HDL & High CAC Score
n=41

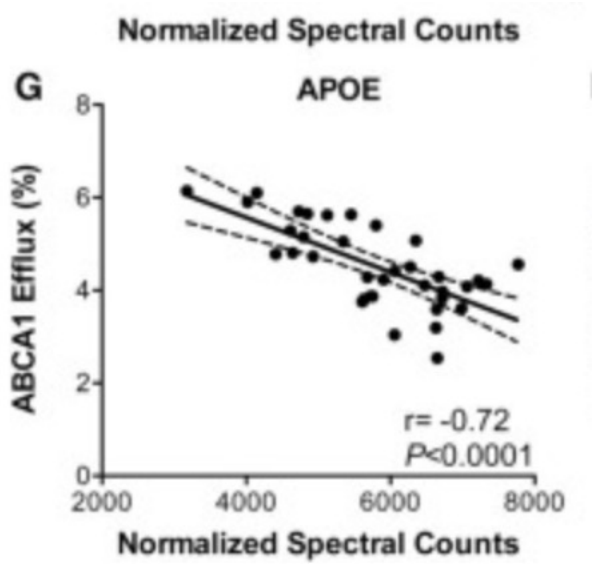
3
High HDL & Low CAC Score
n=15

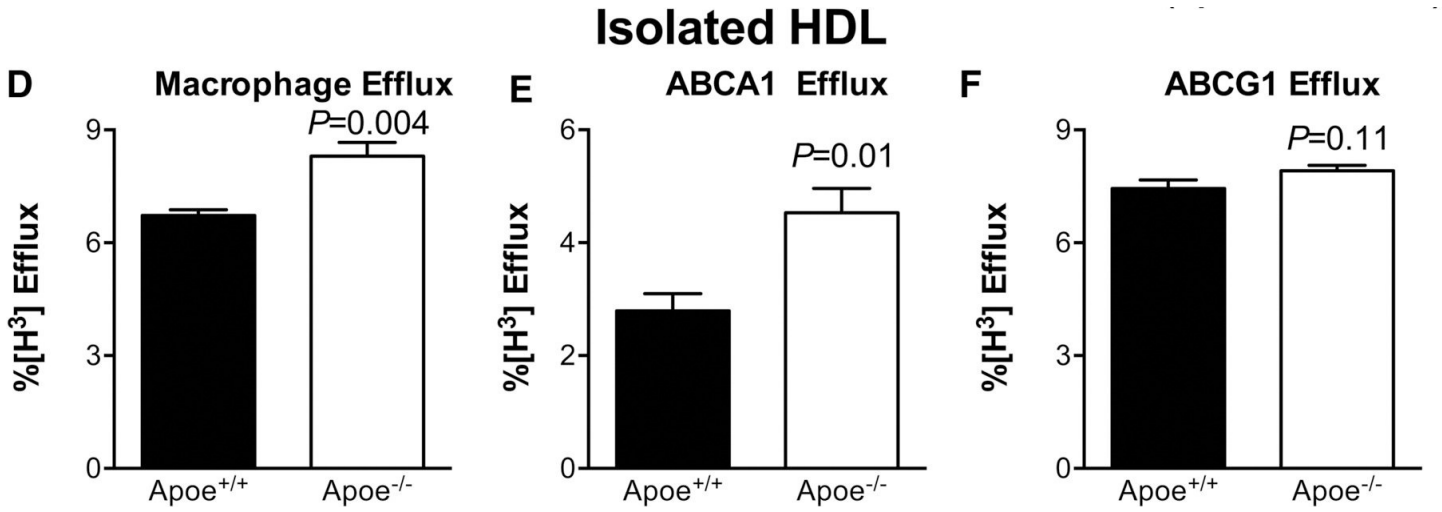
4
High HDL & High CAC Score
n=10



P=0.522
R^2=0.0125
P=0.211
R^2=0.0408
P=0.1066
R^2=0.219
P=0.1428
R^2=0.202

APOE deficient HDL has Increased Sterol Efflux





Pamir, JLR, 2016

APOE vs Sphingomyelins

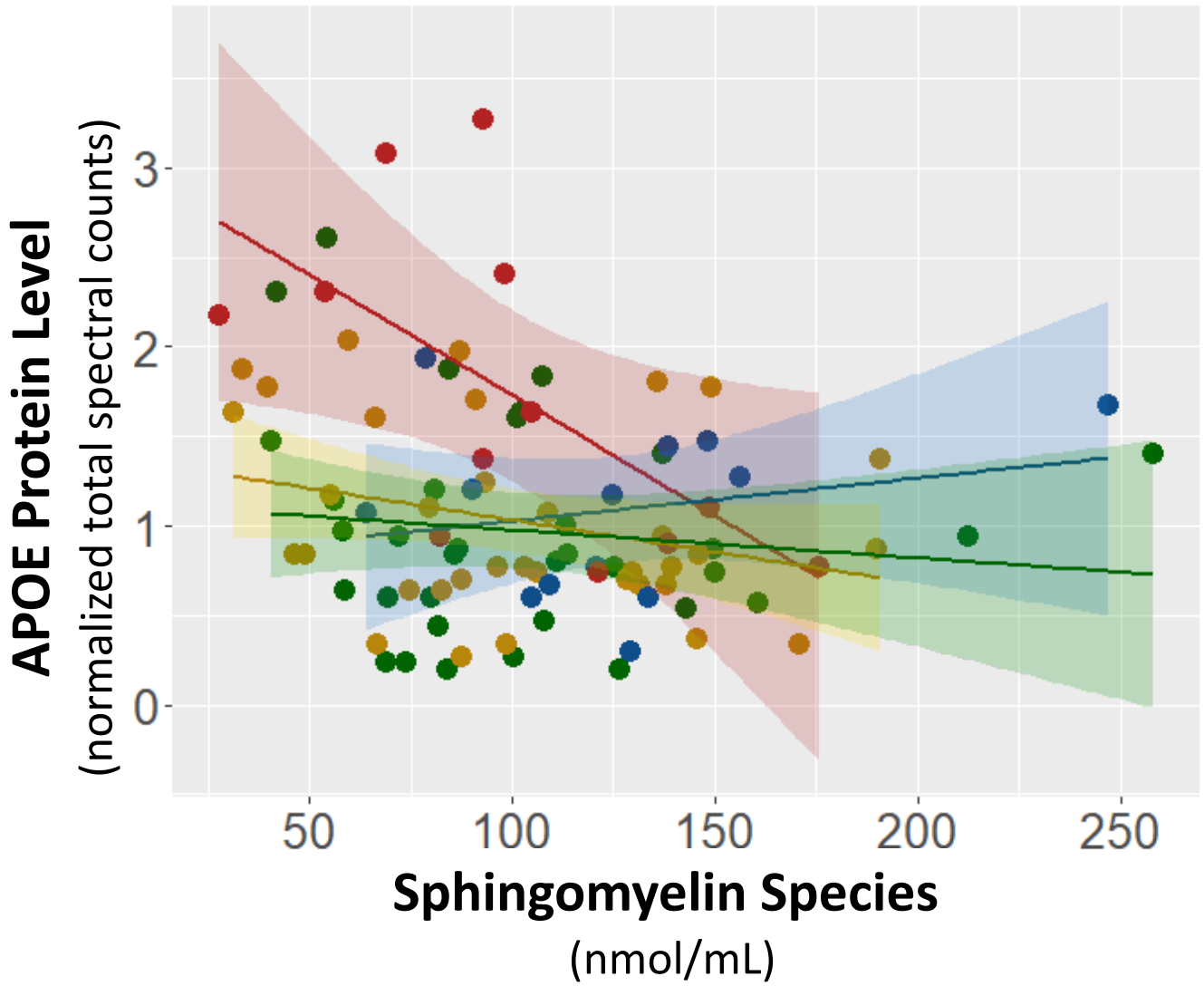
NHDL / LCAC
NHDL / HCAC
HHDL / LCAC
HHDL / HCAC

1
Normal HDL & Low CAC Score
n=36

2
Normal HDL & High CAC Score
n=41

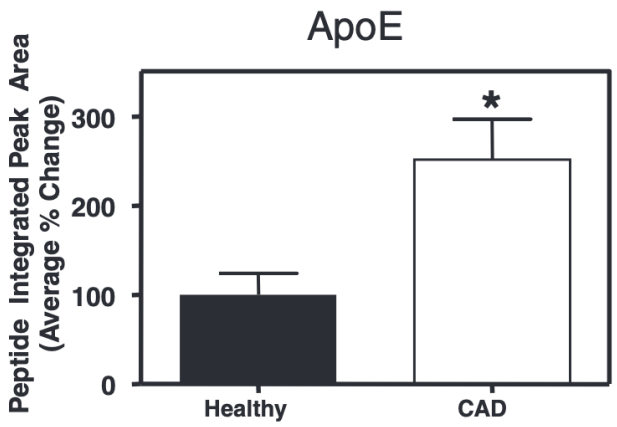
3
High HDL & Low CAC Score
n=15

4
High HDL & High CAC Score
n=10

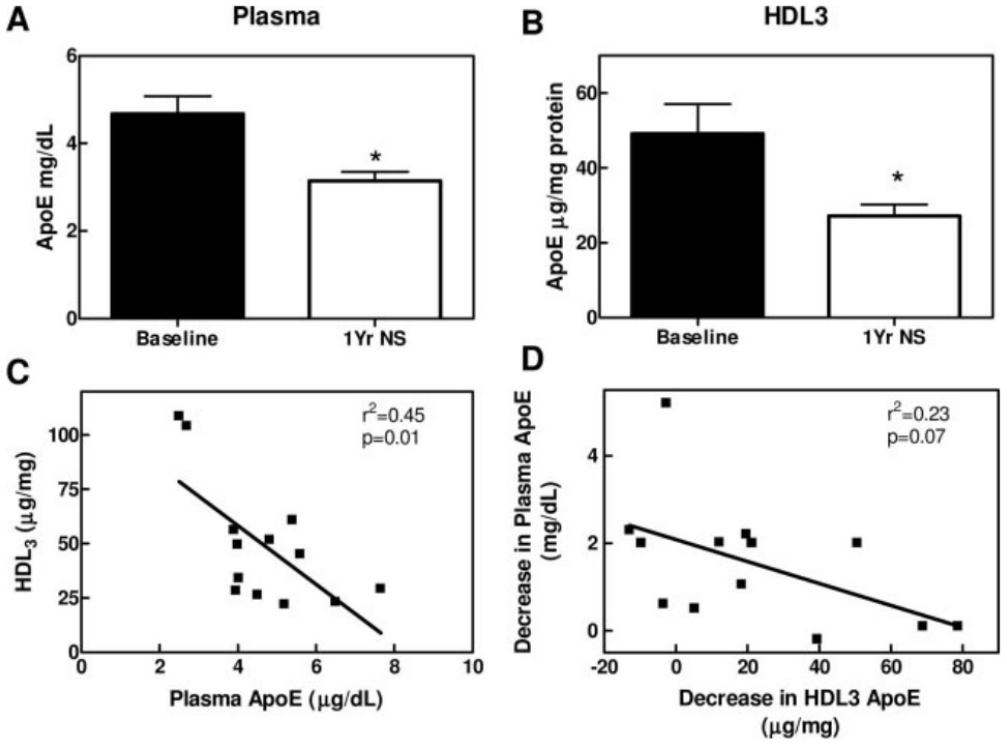


P=0.496
R^2=0.0146
P=0.0926
R^2=0.0833
P=0.459
R^2=0.0508
***P=0.0324**
R^2=0.381

APOE is increased on HDL of CAD subjects



Statin+Niacin Treatment Reduces HDL Associated APOE



Green, Circulation, 2008

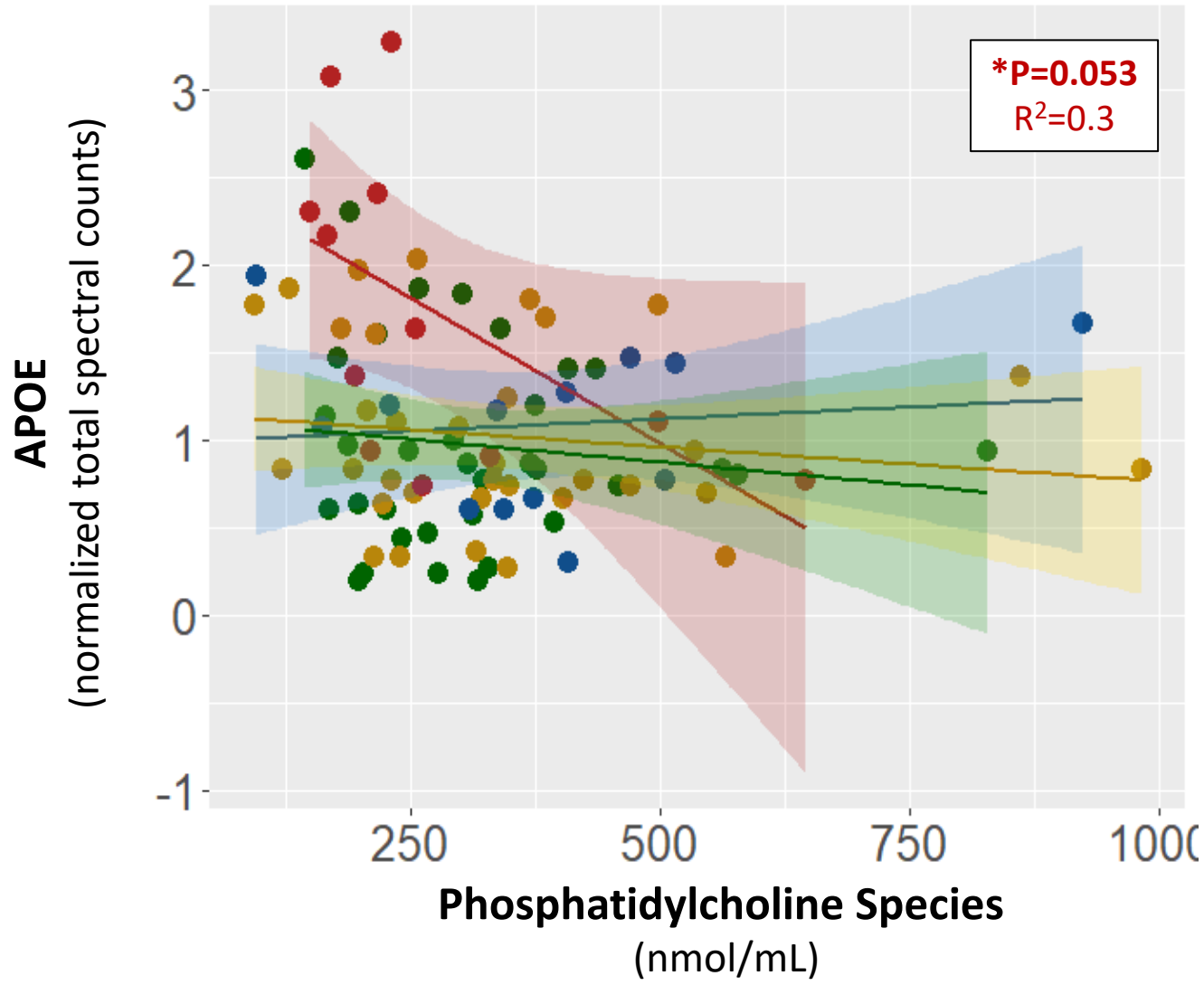
APOE Inversely Associates with PC

1
Normal HDL &
Low CAC Score
n=36

2
Normal HDL &
High CAC Score
n=41

3
High HDL &
Low CAC Score
n=15

4
High HDL &
High CAC Score
n=10



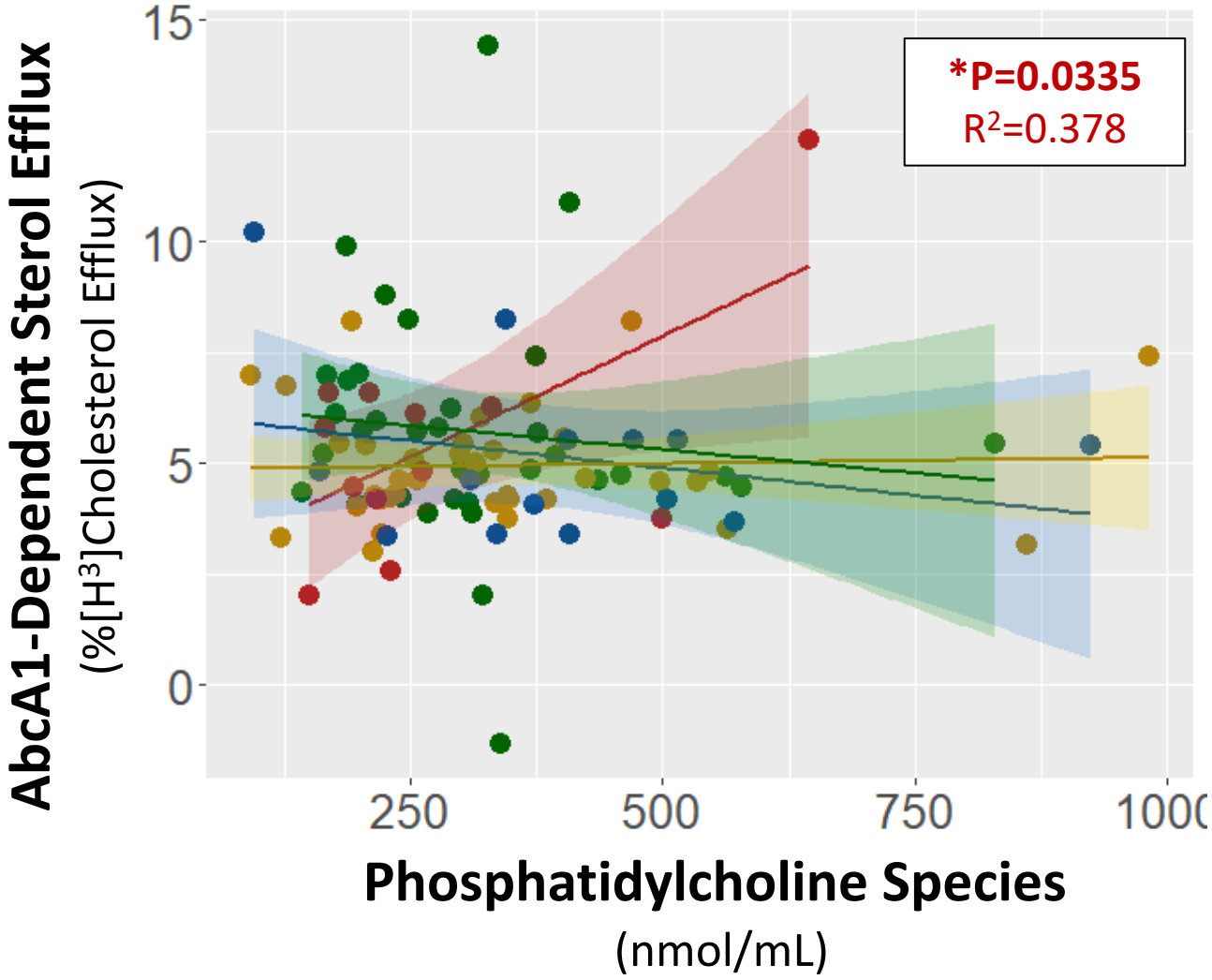
PC Directly Associates with Sterol Efflux

1
Normal HDL &
Low CAC Score
n=36

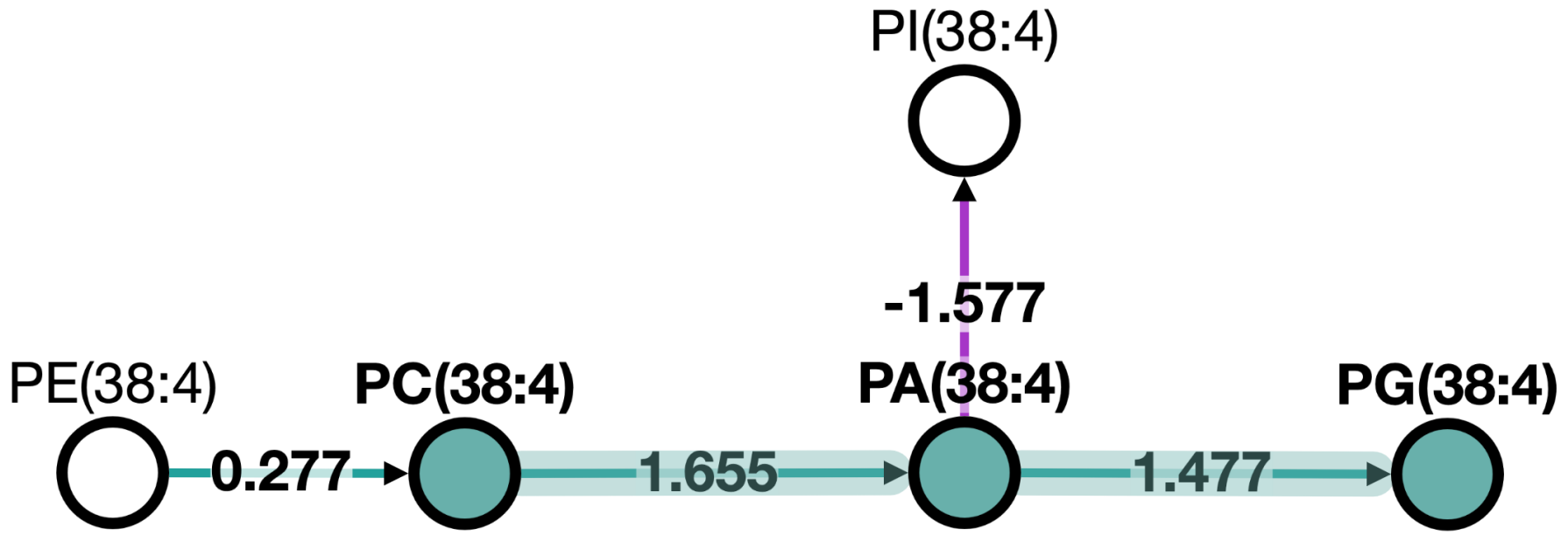
2
Normal HDL &
High CAC Score
n=41

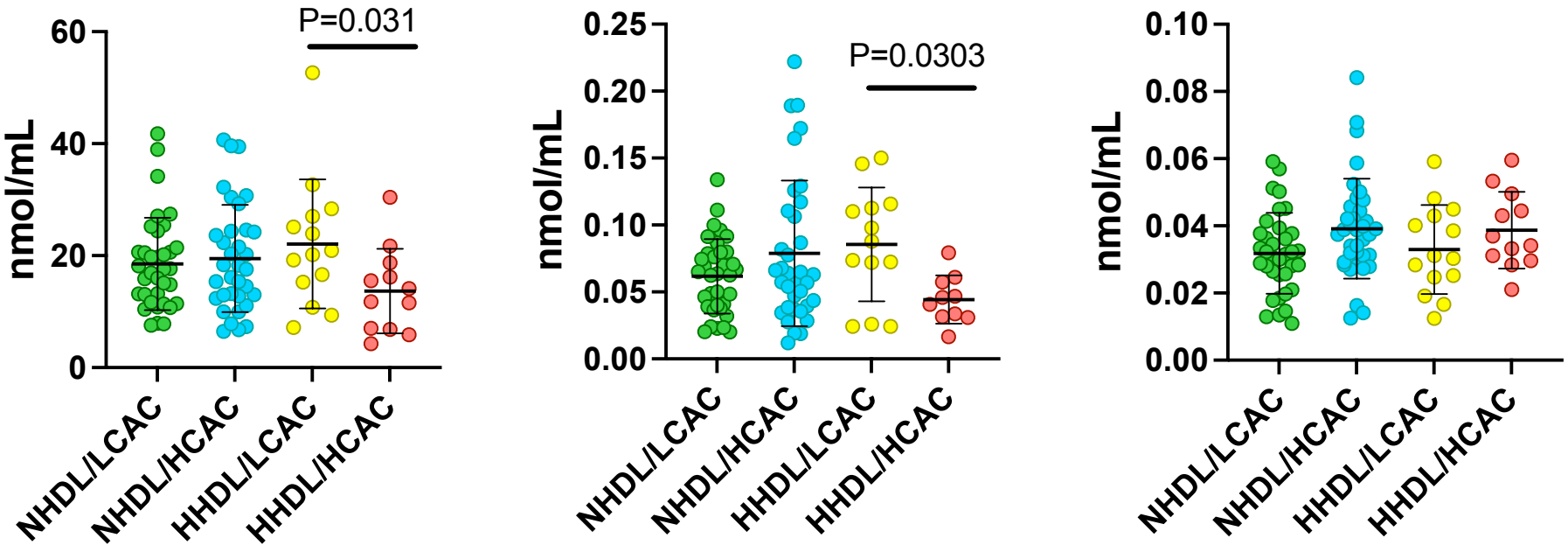
3
High HDL &
Low CAC Score
n=15

4
High HDL &
High CAC Score
n=10

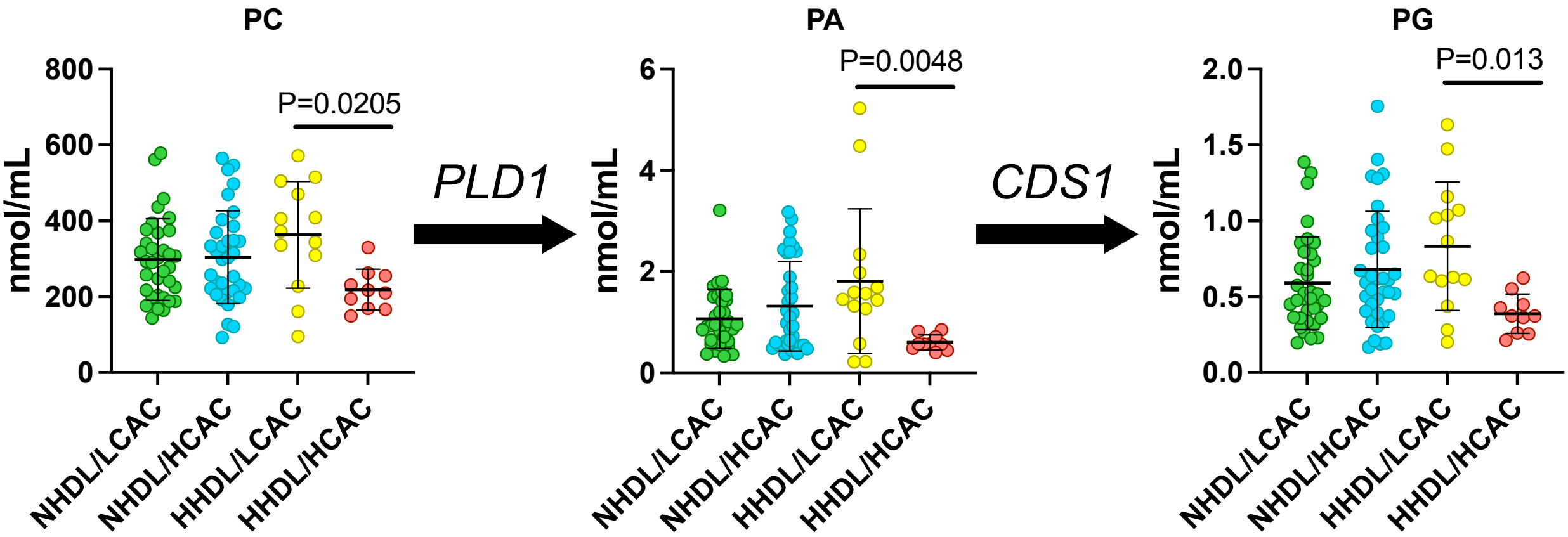


High HDL/High CAC vs Normal HDL/Low CAC: Increased Conversion of PC to PA to PG



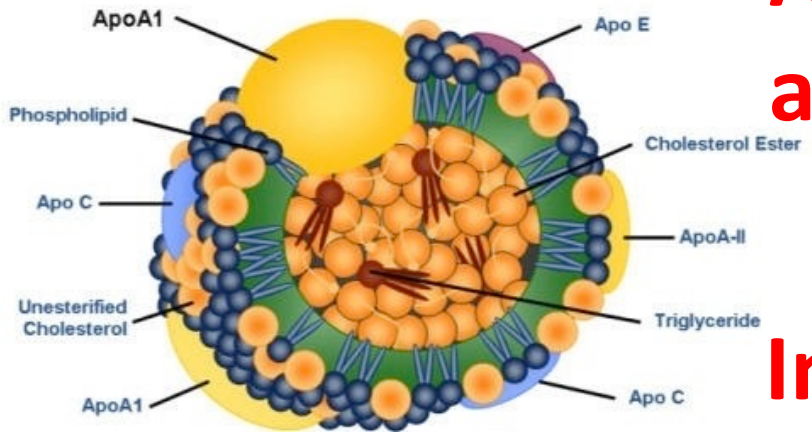


PLD1 and CDS1 drive the conversion of PCs to PGs



Dysfunctional HDL is Linked to Disease

Functional HDL Particle



Increased APOE association

Increased conversion of PC to PA to PG

Dysfunctional HDL Particle

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NIH/NHLBI R01