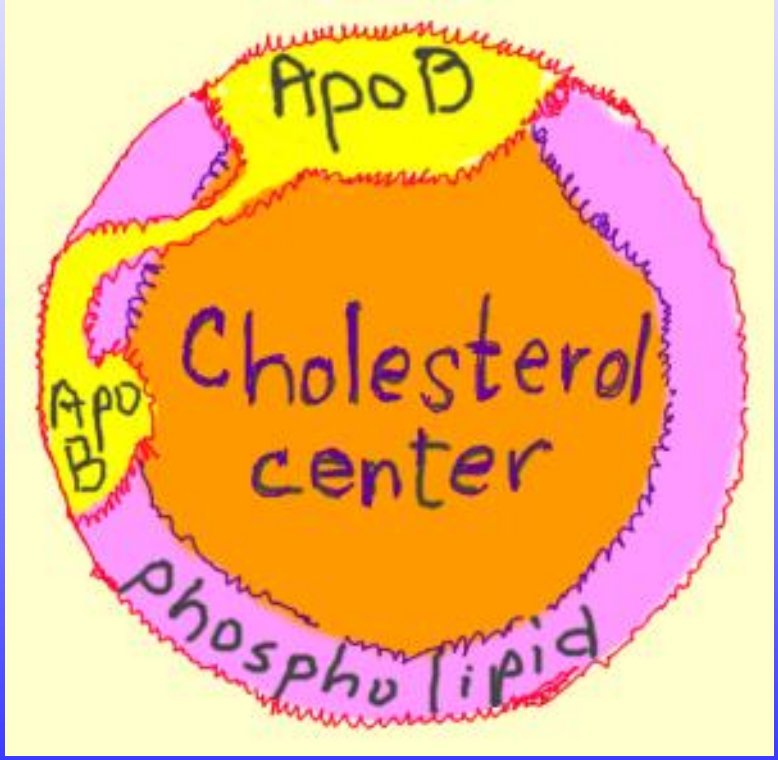
Validation of an ELISA development kit for apolipoprotein B measurement in dried blood spots



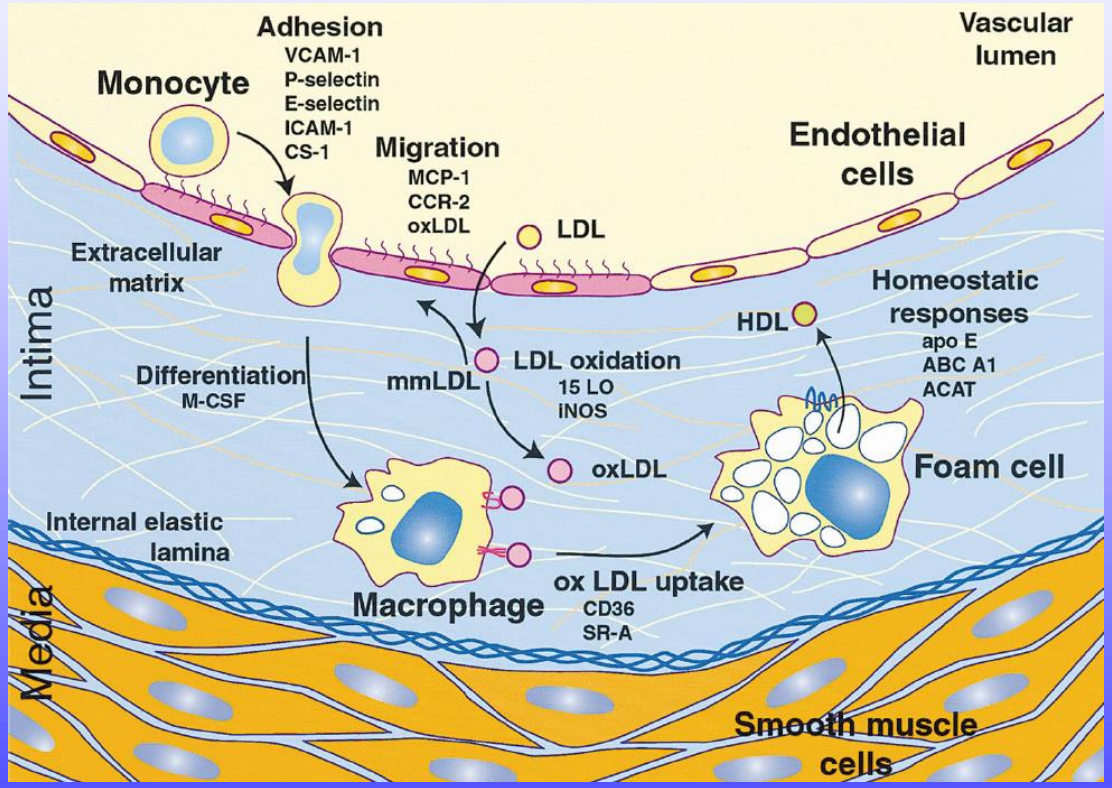
Geeta N Eick, Paul Kowal, J Josh Snodgrass

OREGON

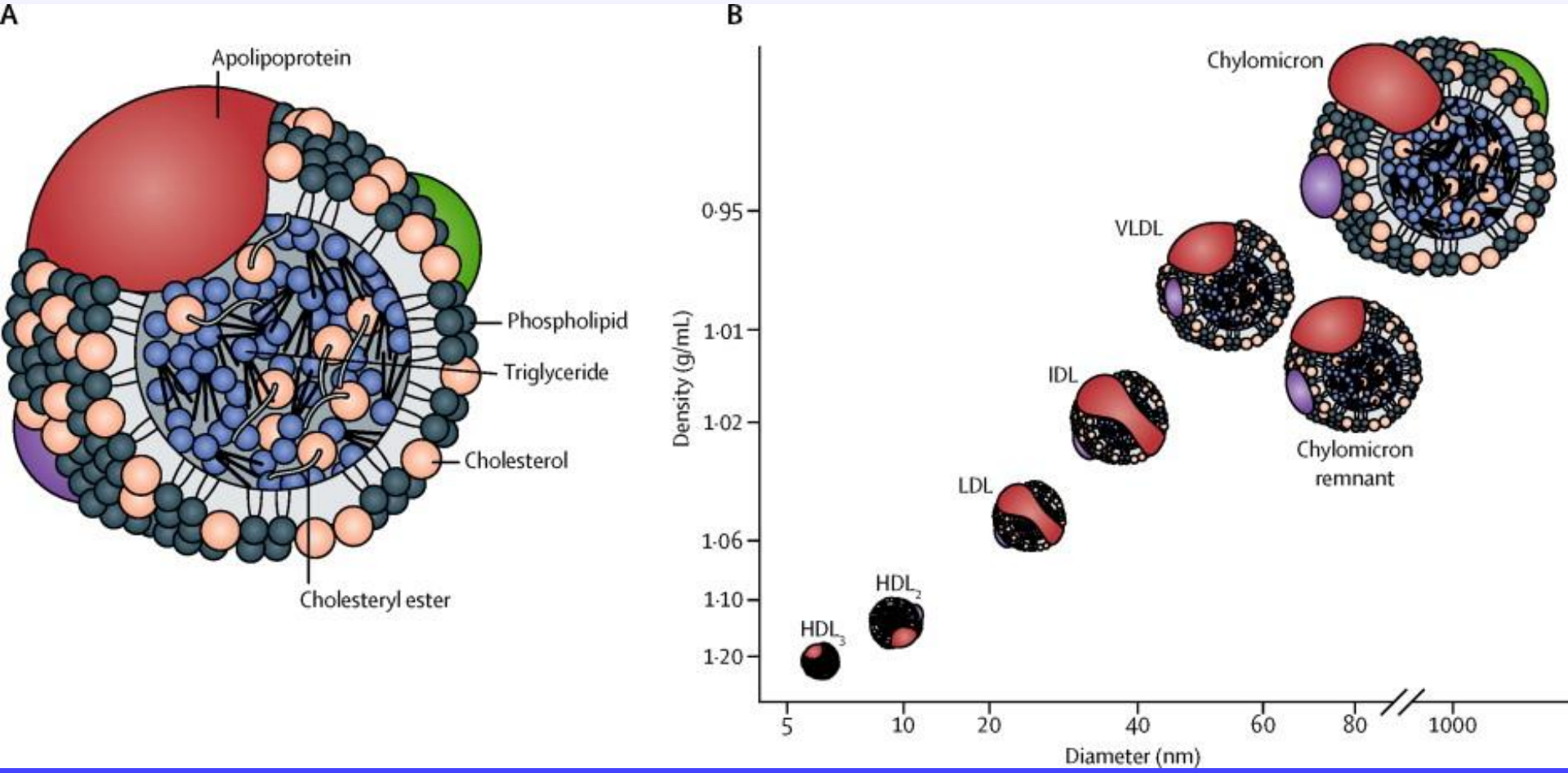
Ischemic Cardiovascular Disease

- Leading cause of death worldwide in both developed and developing countries
- Age-standardized death rates due to ICD have declined steeply since 1980 in developed nations, but rates in Eastern Europe, Central Asia, are increasing
- 90% of ICD cases estimated to be preventable; predicting risk of ICD development therefore critical!
- Primary cause of ICD is atherosclerosis; chronic inflammatory state

Lipoproteins & atherosclerosis



What are Lipoproteins?



Markers of Ischemic Cardiovascular Disease

- Standard markers of CVD:
  - LDL-cholesterol (LDL-C)
- Overwhelming evidence that ApoB superior marker of CVD risk

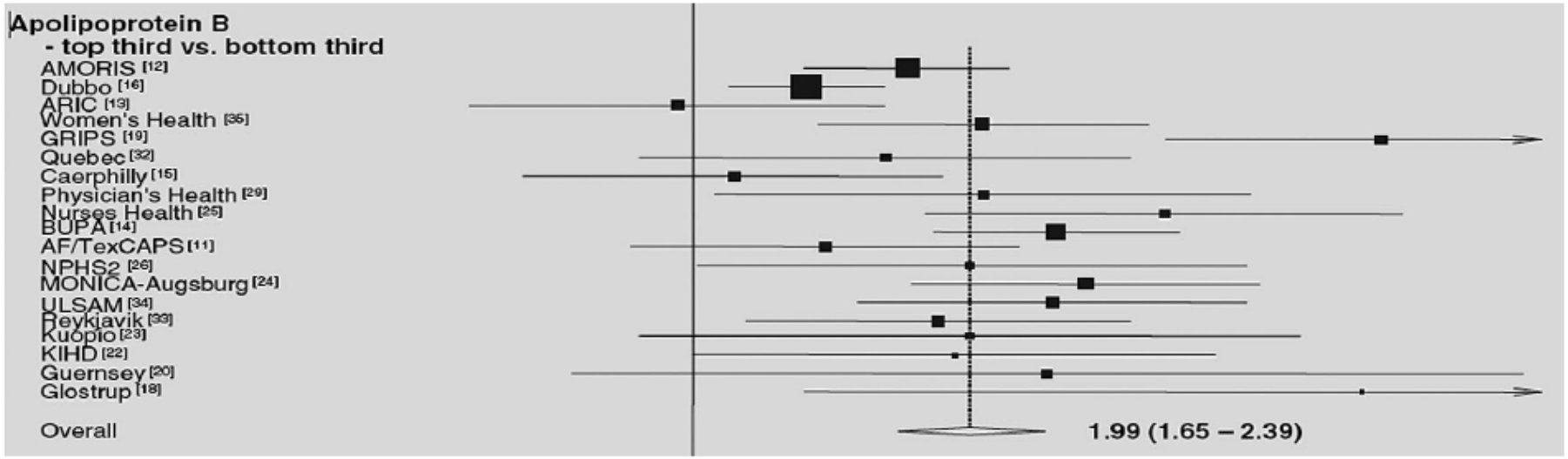


Fig. 1. Relative risk of CHD [adapted from Thompson and Danesh *(37)*].

1 copy of ApoB per LDL molecule

Importance of a 1:1 ApoB: LDL molecule ratio

- LDL cholesterol content can vary considerably among individuals with the same LDL particle concentration
- The higher the actual number of dense LDL particles, the greater the risk of CVD development
- American Diabetes Association, American College of Cardiology issued a joint consensus statement in 2008 that ApoB be the final test of the effectiveness of any LDL-cholesterol-lowering treatment

Other advantages of ApoB

- More amenable to standardization than LDL-C measurement
- ApoB standardization has been achieved by an IFCC standardization project
- Almost all ApoB is bound to lipoproteins with no measurable concentration of ApoB in aqueous medium
- Levels of ApoB are not affected by food ingestion, therefore it can be measured in the non-fasting state

Prior DBS assays for ApoB

- Vladutiu et al. 1980 – Electrodiffusion
- Dudman 1985 – Radial immunodiffusion
- Ohta et al., 1988 – ELISA
- Micic et al., 1988 – double rocket immunoelectrophoresis

Cumbersome assays, used antibodies or antisera produced in-house or that are no longer available, or both.

Mabtech ApoB ELISA development kit

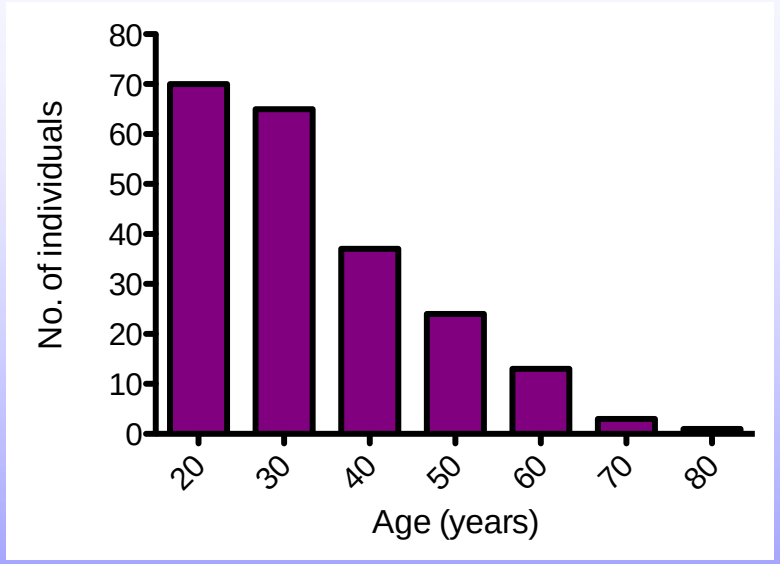
- Monoclonal antibody (capture) 20/17
- Biotinylated monoclonal antibody (detection) LDL 11
- Streptavidin-horseradish peroxidase
- *Purified ApoB standard
- Assay range: 8-800 ng/mL

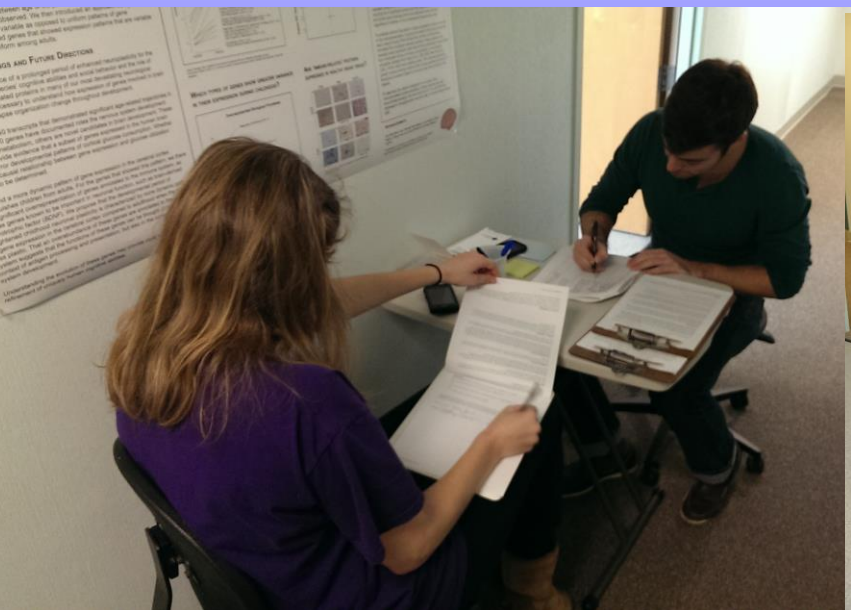
DBS standards & controls

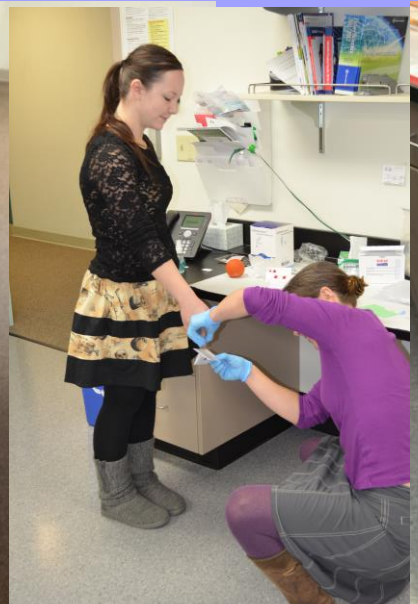
- Purified apoB standard not stable when used to make DBS standards
- Liquichek Lipids Control Level 2
  - ApoA1
  - **ApoB**
  - HDL cholesterol
  - LDL cholesterol
  - Total cholesterol
  - Triglycerides

Eugene200 validation samples

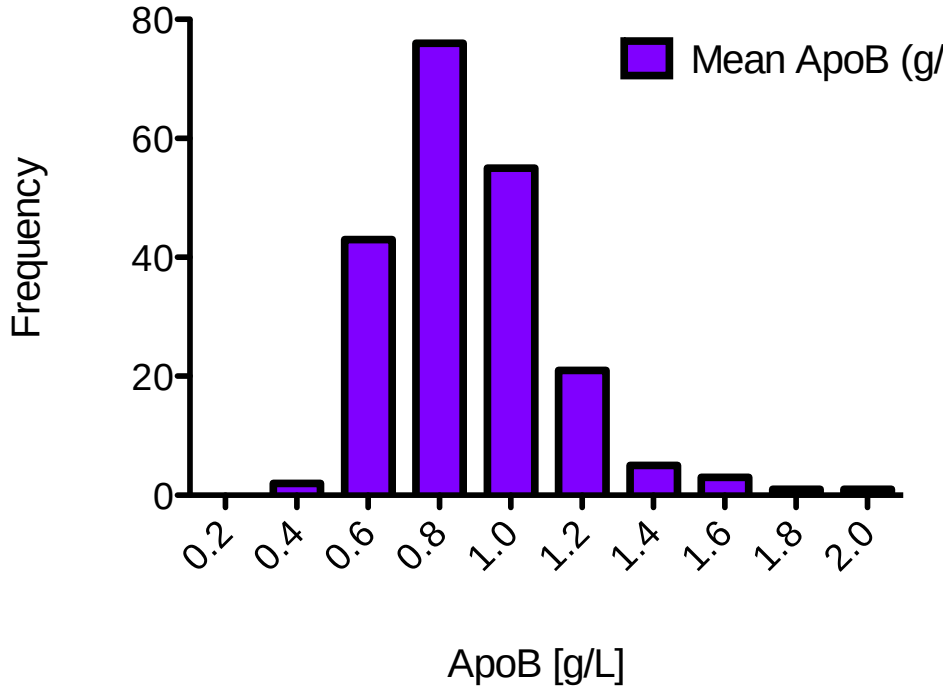
- Recruited 208 adults from Eugene & Springfield, OR from Nov 2014 - Feb 2015
- Collected matched fingerprick DBS, venous DBS, plasma, buffy coat, & saliva samples

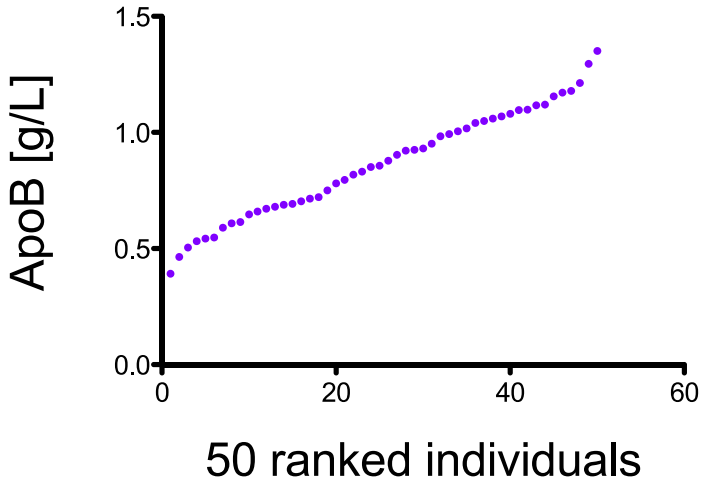






Selected 50/208 samples with vDBS apoB values spanning the range of observed values



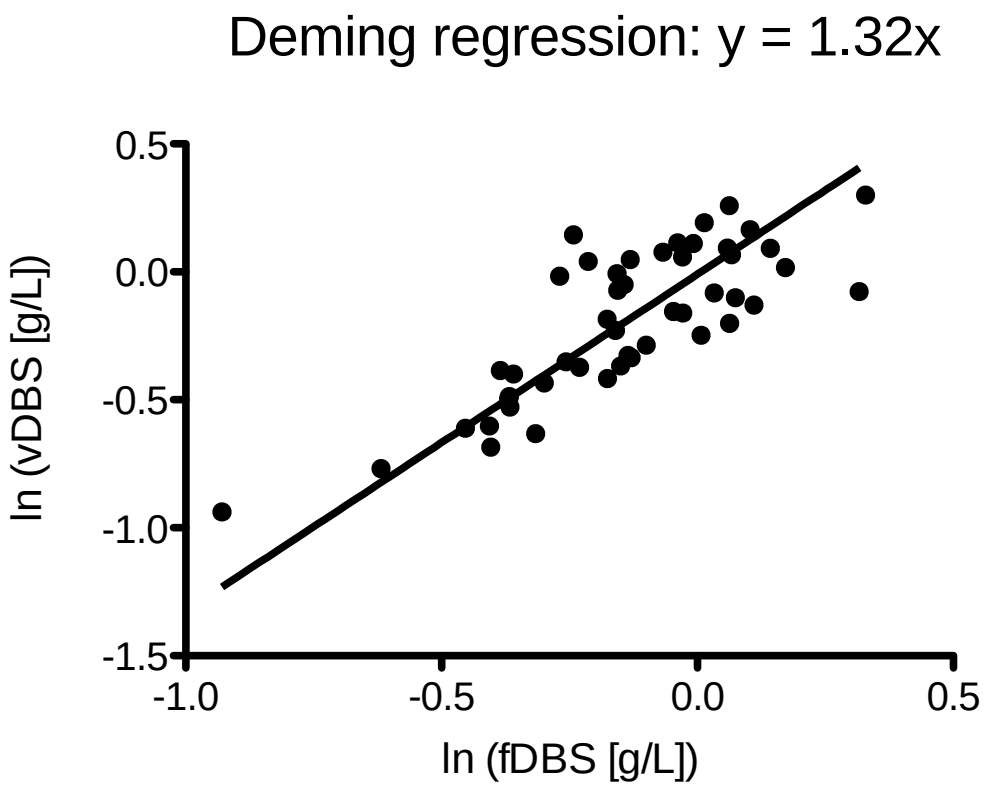


< 1.04 g/L – low risk coronary artery disease
1.22 - 1.4 g/L – high risk
> 1.4 - very high risk

Stats for ApoB DBS sandwich ELISA

- Intra-assay CV:
  - High control: 1.33%
  - Low control: 1.08%
- Inter-assay CV: 11.2%
- Analytical Sensitivity: 0.02 g/L (n = 12 plates)

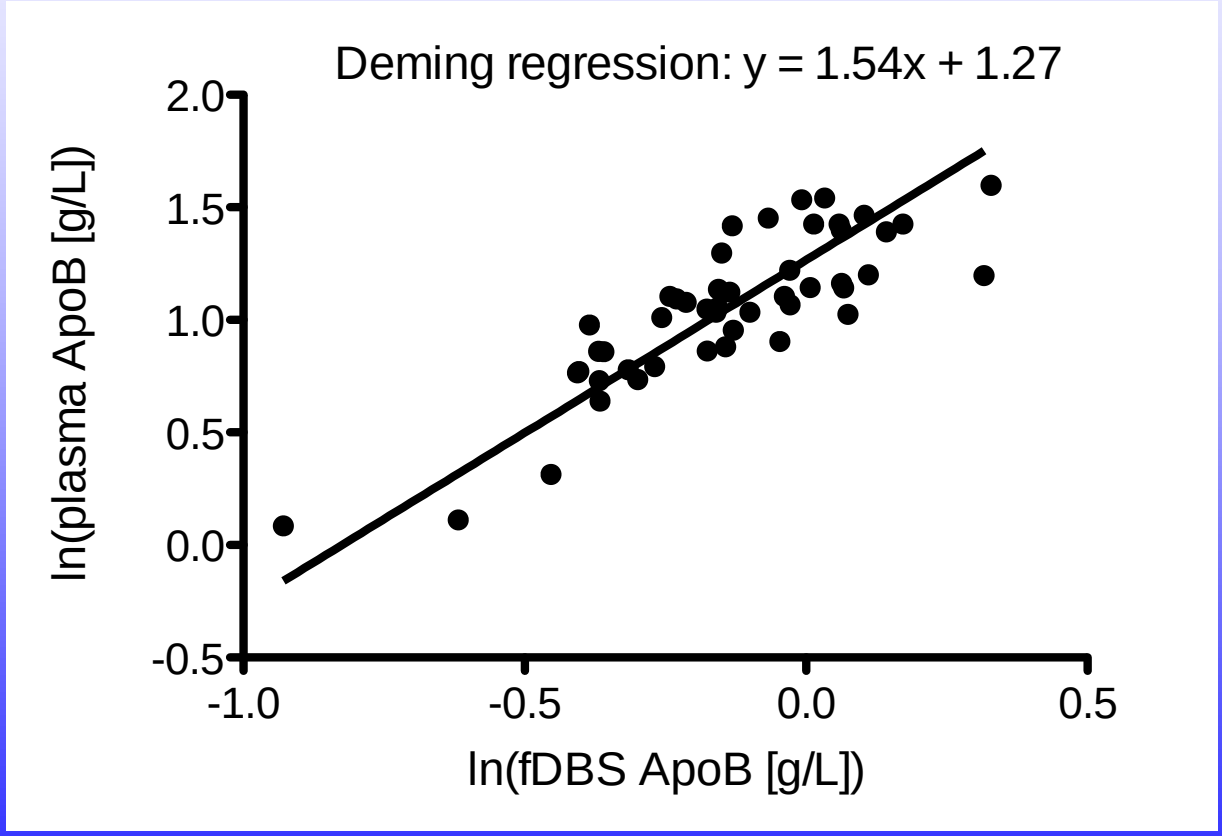
No significant difference between vDBS and fDBS ApoB measurements



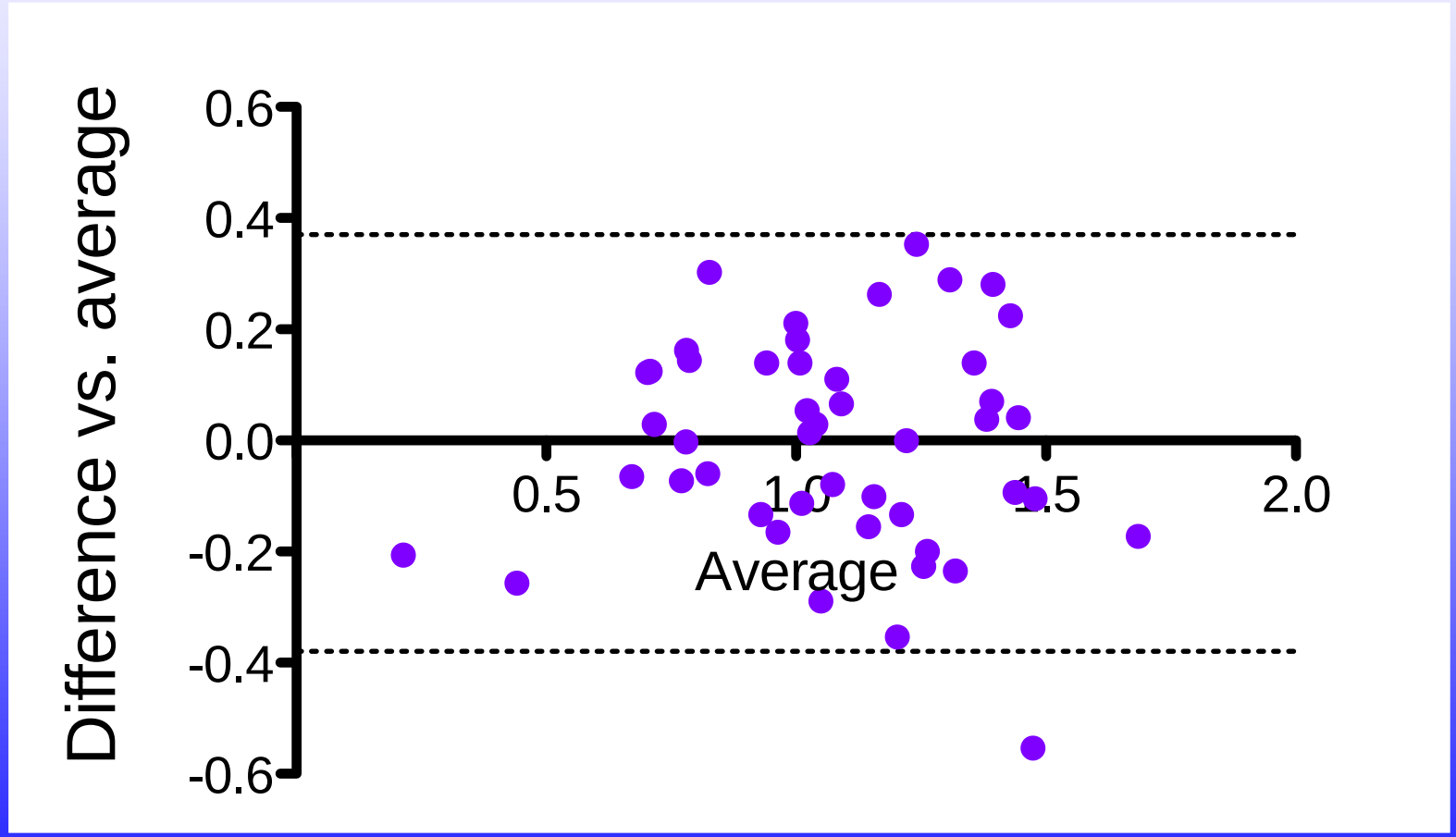
95% CI slope: 1.0 - 1.6

- No proportional difference between vDBS and fDBS

Significant linear relationship between fDBS ApoB values & plasma ApoB values



No bias (-0.005) and very few outliers based on Bland-Altman analysis



Linearity

Linearity in acceptable range between 80-120% (but would ideally like between 90-110%)

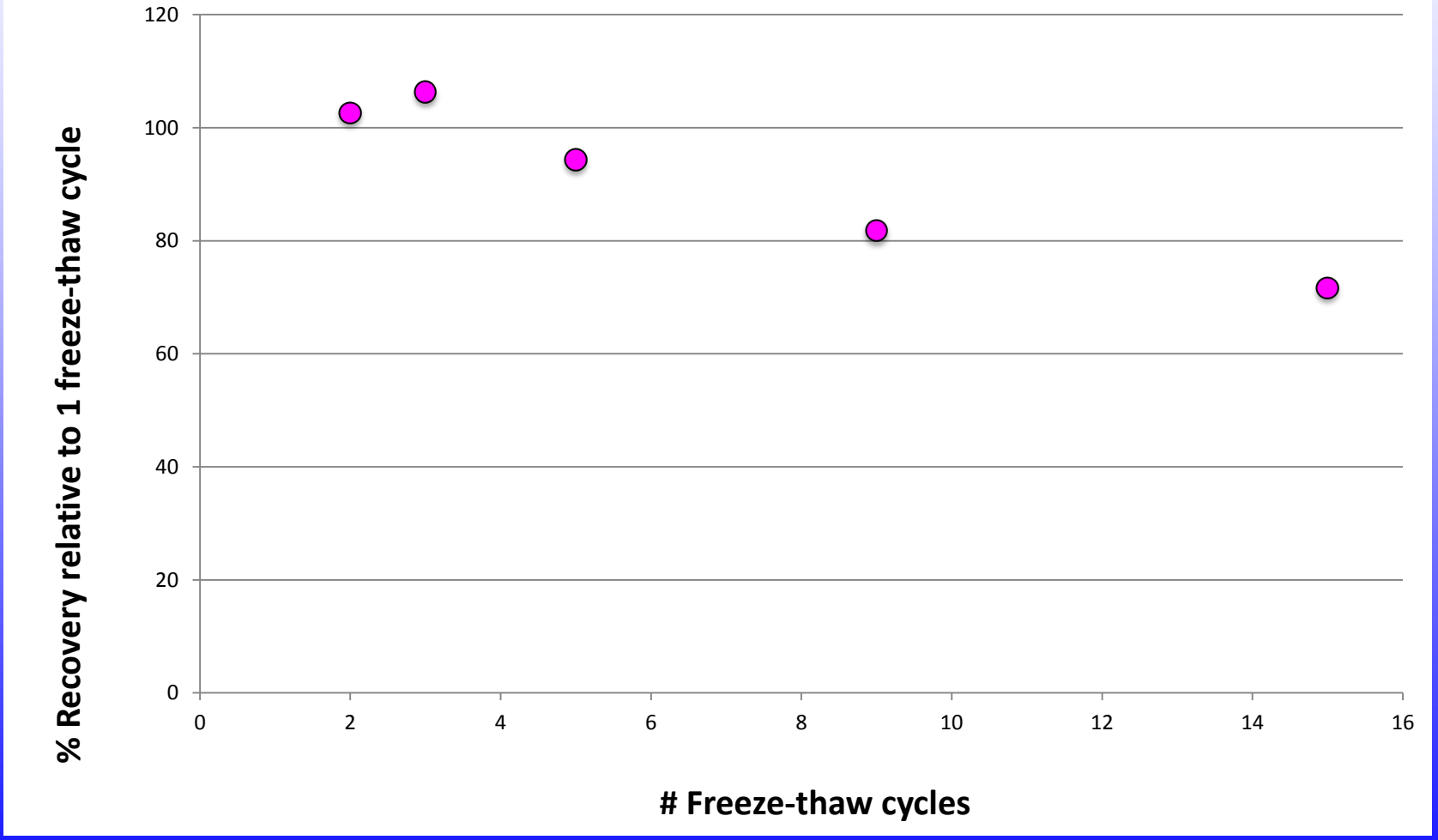
Dilution		vDBS (n=6)
1:2	Average % of expected	**102**
	Range (%)	**92-109**
1:4	Average % of expected	**109**
	Range (%)	**98-117**
1:8	Average % of expected	**116**
	Range (%)	**106-123**

Spike & Recovery

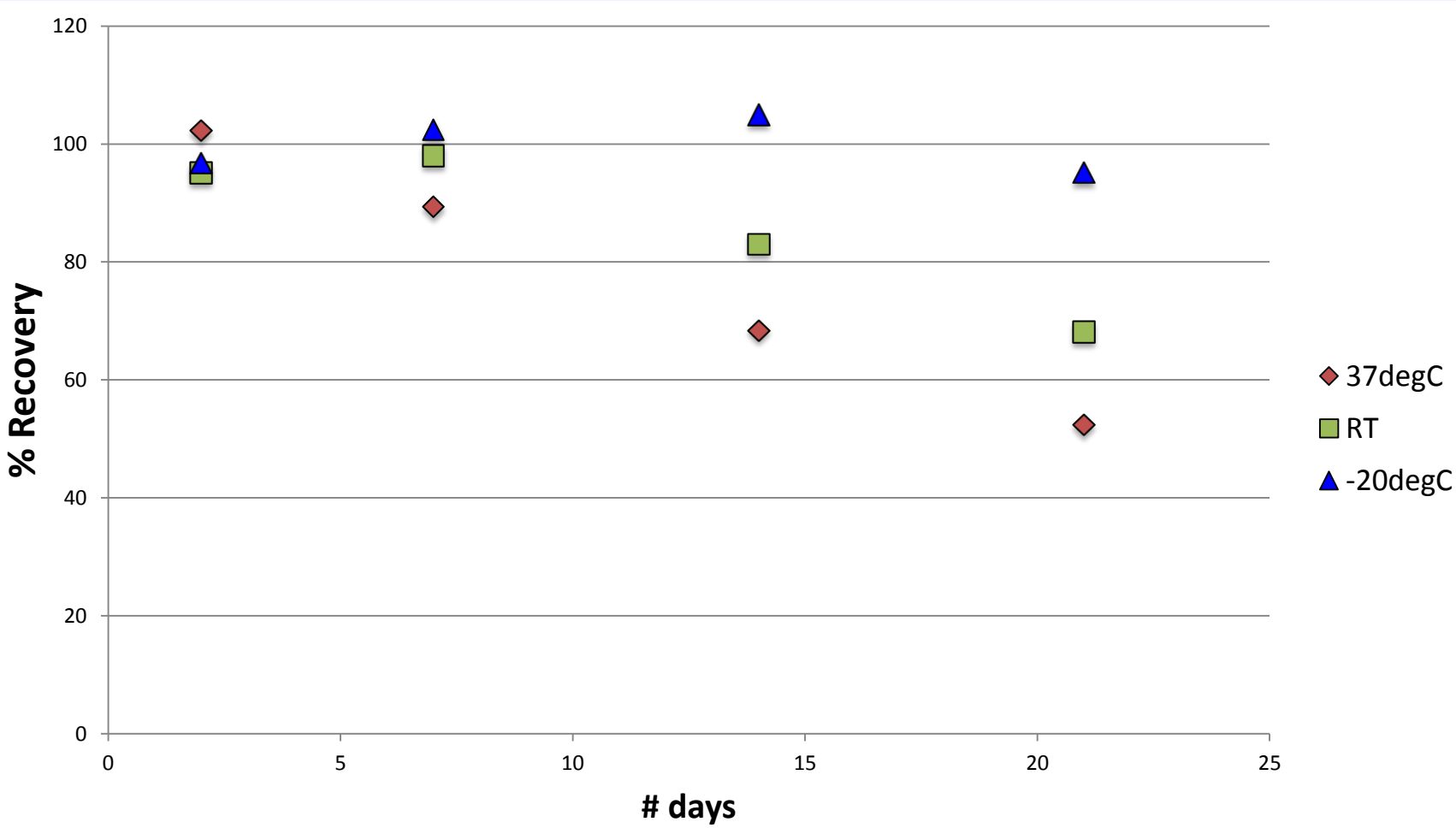
Sample	Observed (mg/dL)	Expected (mg/dL)	% Recovery
#1	117.61	113.64	88.0
#2	128.10	127.64	100.4
#3	147.9	141.71	104.4
#4	117.7	119.14	98.8
#5	102.25	100.6	101.6

DBS matrix does not appear to interfere with analyte recovery

ApoB concentrations decrease with increasing number of freeze-thaw cycles



ApoB concentrations decreased at storage temps > -20°C



Physiological range of hematocrit had no effect on ApoB concentration

Hematocrit %	% Recovery (vs. 50% hematocrit)
30	118%
40	126%
60	100%
80	112%

Limitations

- No “gold standard”
  - Obtained plasma quality controls from Northwest Lipid Metabolism And Diabetes Research Laboratories (repository for WHO/IFCC reference material for apo B)
- Assay involves numerous dilution steps

Conclusions

- DBS-based ELISA assay for ApoB appears promising for population-level research
- Simple to perform, & can stockpile reagents
- Further investigation of assay linearity
- Assess performance with reference laboratory QC standards

Acknowledgements

- Biomarker network meeting organizers
- Liz Thiele

Eugene200 team: Lauren Moore, Melissa Liebert, Theresa Gildner, Josh Shrock, Tyler Barrett, Elisabeth Goldman, Tyler Fording, Devan Compton, Anna Hanson, Micaela Burns, Blanche Blumenthal, Colin Lipps, Robyn Brigham, Haley Brown, Sophie McGinley, Brian McCree, Caroline Porter, Molly Turner, Oliver Wald, Zach Clayton

Funding

- NIH NIA Interagency Agreement YA1323-08-CN-0020
- NIH R01-AG034479
- University of Oregon (Bray Fellowship to JJS)