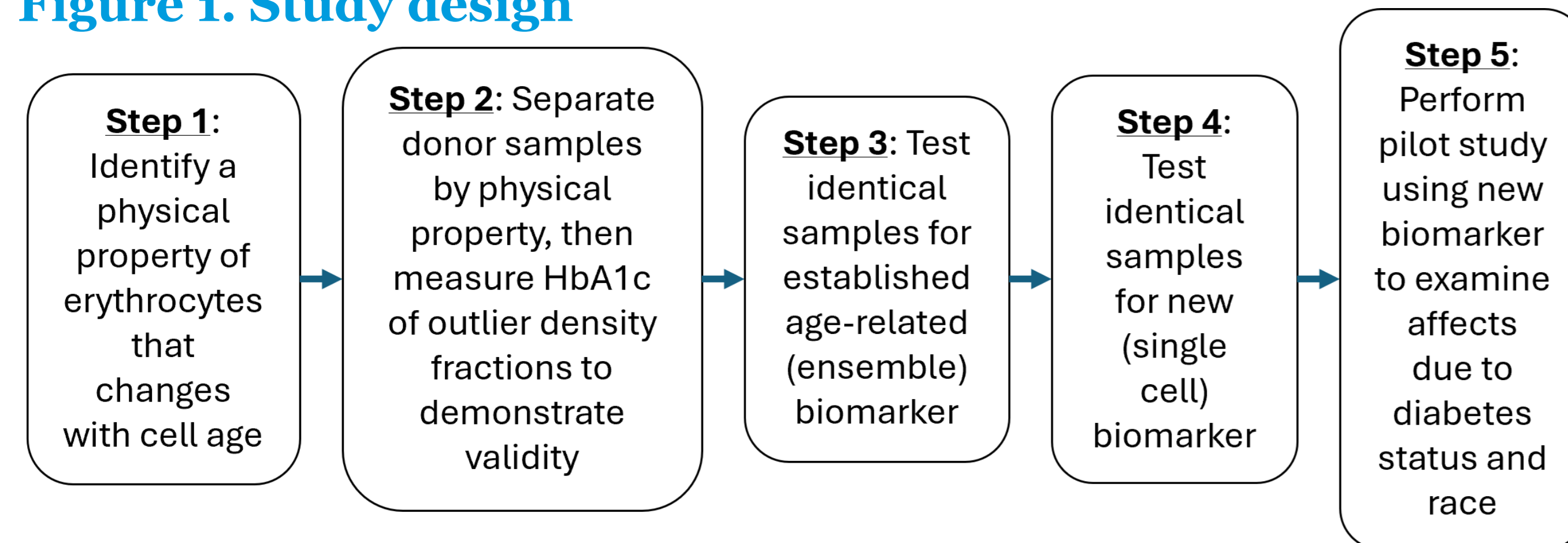


## Background

- Ensemble (laboratory) glycated hemoglobin (HbA1c) measurements can be affected by erythrocyte lifespan<sup>1</sup>.
- Oxidative stress occurs throughout erythrocyte lifespans and is a major factor leading to senescence<sup>2</sup>.
- This study evaluates the profile of reduced glutathione (GSH, an antioxidant) in young vs old erythrocytes as a potential biomarker for cell age to investigate observed discordance between laboratory HbA1c and CGM-derived HbA1c.

## Methods

Figure 1. Study design



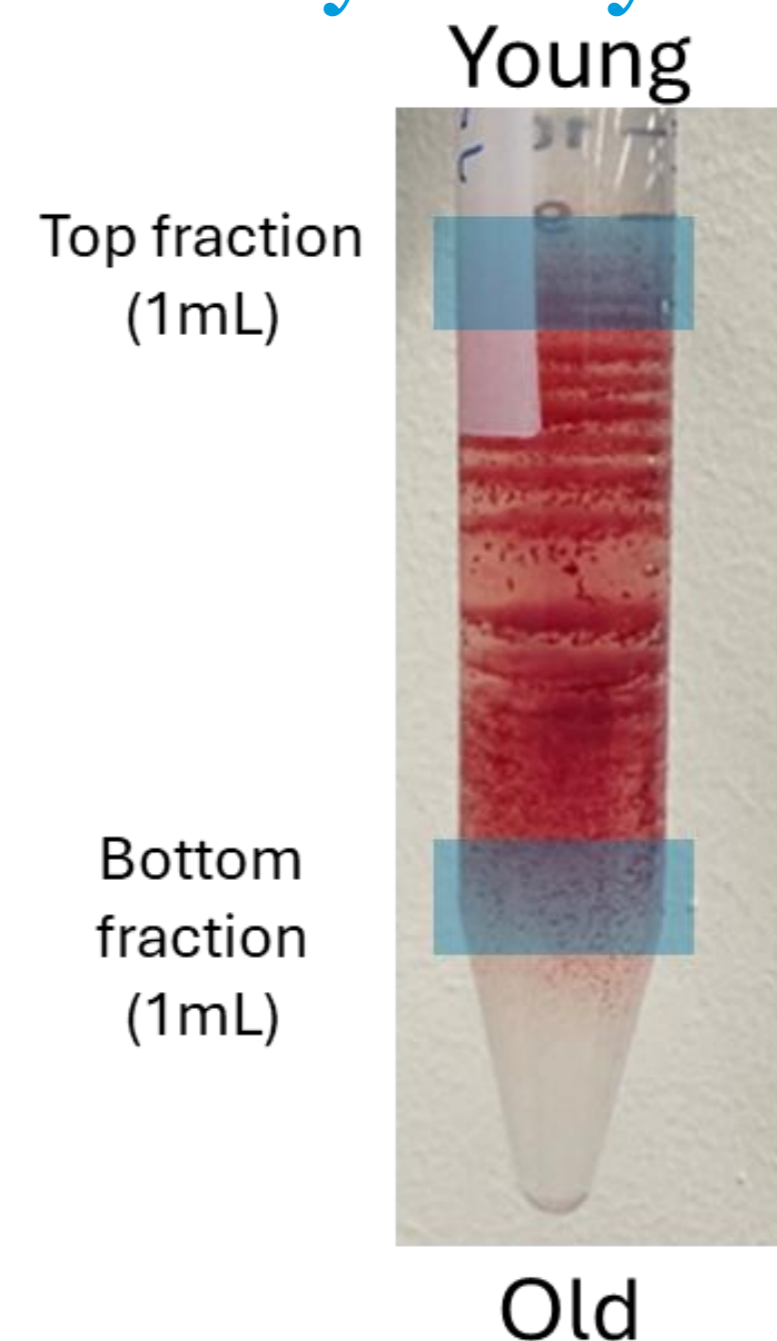
### Study inclusion criteria:

- Donor blood samples: Non-diabetes (A1c < 6.5%) and diabetes (A1c ≥ 6.5%)
- ≥18 years old
- Male and Female
- Caucasian (white) and African American (black)
- N = 117

### Density separation:

- Erythrocytes shrink due to dehydration as they age. We can exploit this and separate them by density using Percoll/centrifugation.
- Percoll density used for continuous gradient formation was 1.105 g/mL.
- Centrifugation performed at 11500 × g for 12 minutes with no brake.

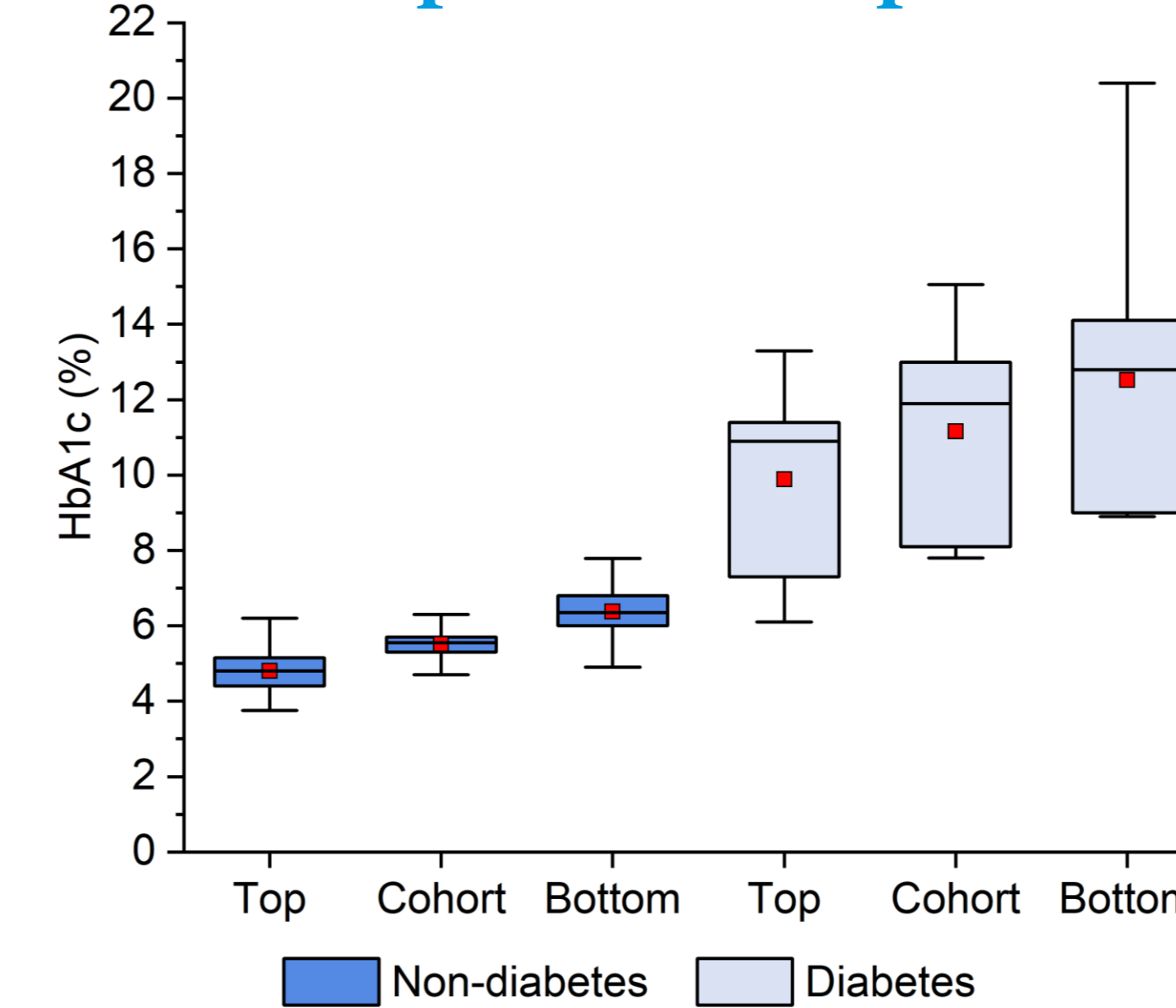
Figure 2. Percoll density separation of erythrocytes



## HbA1c% increases with density

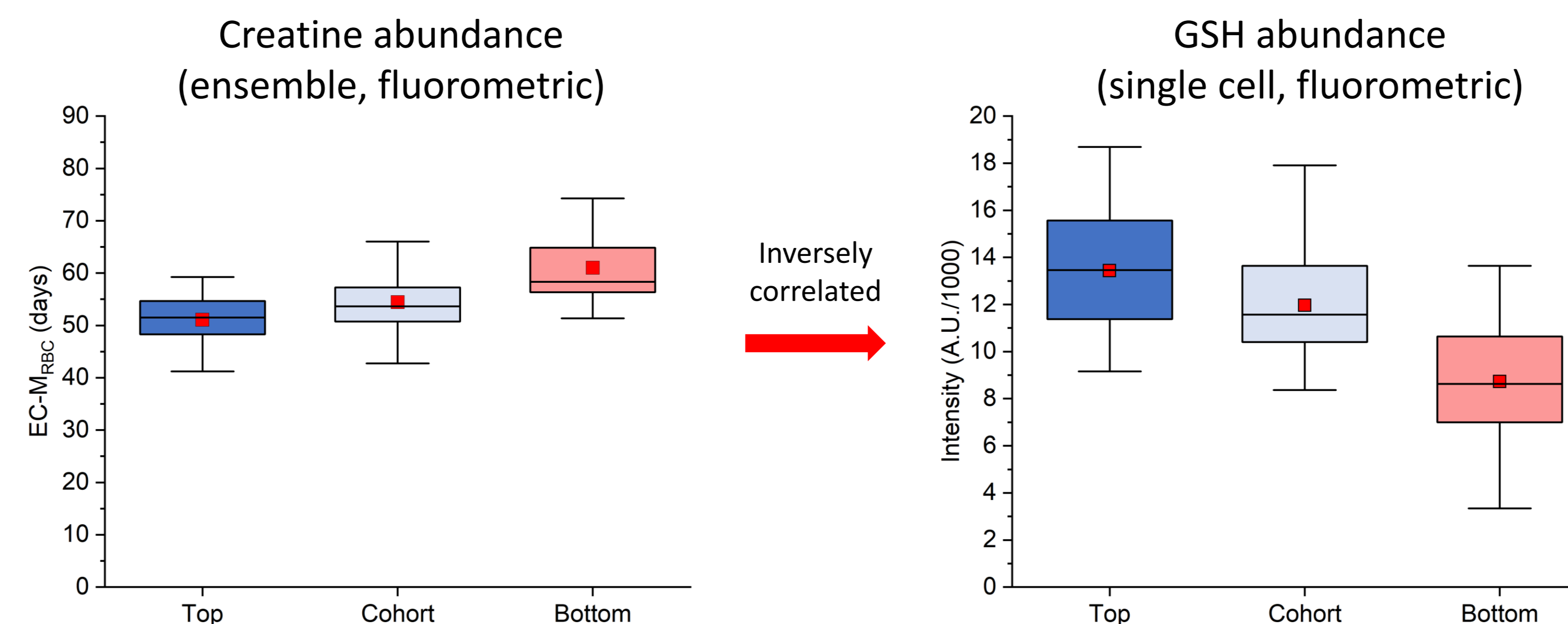
- Erythrocytes from individual donor samples (persons with and without diabetes) were separated by density.
- Top fractions, Cohort (cells not separated by density), and Bottom fractions were collected and run on Abbott Alinity c for HbA1c%.
- A trend amongst all donors is observed: lower HbA1c% in Top fraction (younger), higher HbA1c% in Bottom fraction (older).

Figure 3. HbA1c% from density separated samples



## Correlation is retained between biomarkers

Figure 4. EC-MRBC and GSH abundance plots for density separated samples



- Benchmarking the proposed (single cell) biomarker, GSH, against an existing (ensemble) biomarker, creatine, on identical density-separated donor samples.
- Creatine abundance is known to reduce with cell age and can be used to estimate mean cell age in number of days (EC-M<sub>RBC</sub>). We observed this trend when density separated samples are measured by plate reader<sup>3</sup>.
- Reduced glutathione also shows a relative abundance decrease (intensity in arbitrary units/1000) from top fraction to bottom fraction as measured by flow cytometry.

## Pilot study results

- Mean GSH relative abundance (arbitrary intensity units/1000) and standard deviation were calculated per subgroup.
- Observation #1:** Statistical significance was found between persons without and with diabetes (p<0.01, mean 12.9±2.4 and 11.5±1.2, respectively).
- Observation #2:** All black donor “old cell” fractions statistically differ from all white “old cell” fractions (p=0.03, mean 8.5±2.5 and 9.3±1.9, respectively)
- Observation #3:** Black donors without diabetes “old cell” fractions statistically differ from white donors without diabetes “old cell” fractions (p<0.01, mean 8.1±2.7 and 9.8±2.1, respectively)
- Observation #4:** Statistical significance was observed between black persons with diabetes and white persons with diabetes in their “young cell” fraction (p<0.01, 14.8±1.7 and 13.2±1.6, respectively) and in their “old cell” fractions (p=0.03, 9.5±2.0 and 8.5±1.1, respectively)

## Conclusion

- This study suggests that reduced glutathione is markedly decreased from persons without diabetes to persons with diabetes. If categorized by subgroup, we may be able to indicate shifts in relative mean cell age for donor samples which fall outside of the standard deviation.

## References

- Cohen RM, Franco RS, Khera PK, Smith EP, Lindsell CJ, Ciruolo PJ, Palascak MB, Joiner CH. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. *Blood*. 2008 Nov 15;112(10):4284-91. doi: 10.1182/blood-2008-04-154112. Epub 2008 Aug 11. PMID: 18694998; PMCID: PMC2581997.
- Bogdanova A, Kaestner L, Simionato G, Wickrema A, Makhro A. Heterogeneity of Red Blood Cells: Causes and Consequences. *Front Physiol*. 2020 May 7;11:392. doi: 10.3389/fphys.2020.00392. PMID: 32457644; PMCID: PMC7221019.
- Kameyama M, Koga M, Okumiyama T. A novel method for calculating mean erythrocyte age using erythrocyte creatine. *Aging (Albany NY)*. 2020 May 11;12(9):8702-8709. doi: 10.18632/aging.103193. Epub 2020 May 11. PMID: 32392179; PMCID: PMC7244067.

## Acknowledgements

This study was funded by Abbott.