2023 NIA-Sponsored Biomarker Network Meeting

Harmonization of Four Biomarkers across Nine Nationally Representative Studies of Older Persons

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Cross-national comparisons of biomarker data are challenging

Even excellent labs have machines and assay reagents that vary

In certified laboratories the absolute value of analytes can cover a large range.

For comparisons across countries and over time, harmonization is necessary.

Harmonization Protocol:

Produce blood samples at University of Washington (Reference Lab) to be used in nine study labs

- **Replicate sets of liquid and dried samples were created from serum and plasma from blood banks and from blood remaining after clinical analyses**
- **Three types of materials collected**, i.e., three liters of serum, one liter of plasma and two liters of whole blood and were used to create the approximate 5,200 project samples.
- **UW created a set of plasma, serum, whole blood, and DBS samples** reflecting the range of values for each of the 4 analytes to support optimal cross-calibration algorithms.
    - *CRP: 0 – 30 mg/L*
    - *HbA1c: 3.4% – 14.0%*
    - *Total cholesterol: 0 – 400 mg/dL*
    - *HDL cholesterol: 0 – 130 mg/dL*

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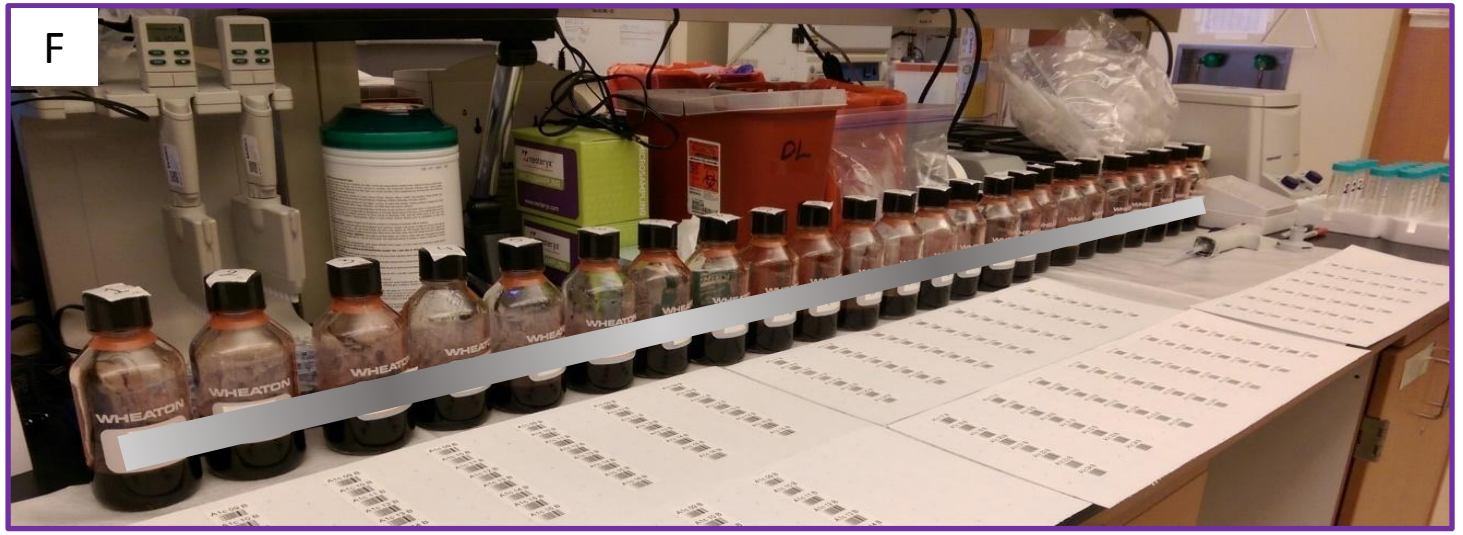


A set of 24 HARMONIZATION samples for each analyte were shipped to each laboratory with dry ice by World Courier

Laboratories assayed samples twice

Assay results used to create equation calibrating study lab results to UW "reference" results

Coefficients from these equations for each laboratory were then applied to the logged raw data from the study population to estimate harmonized versions of the study data:

Studies Included in Harmonization

Country	Study	
China	CHARLS	China Health and Retirement Longitudinal Study
England	ELSA	English Longitudinal Study of Ageing
Brazil	ELSI-Brazil	Brazilian Longitudinal Study of Aging
United States	HRS	Health and Retirement Study
Indonesia	IFLS	Indonesia Family Life Survey
India	LASI	Longitudinal Aging Study in India
India	LASI-DAD	Longitudinal Aging Study in India – Diagnostic Assessment of Dementia Study
Northern Ireland	NICOLA	Northern Ireland Cohort for the Longitudinal Study of Ageing
Ireland	TILDA	Irish Longitudinal Study on Ageing

Blood Sample Types

Country	Study	Blood Sample Type			
		Whole blood	Plasma	Serum	Dried blood spots
China	CHARLS		X		
England	ELSA	X		X	
Brazil	ELSI-Brazil	X		X	
United States	HRS			X	
Indonesia	IFLS				X
India	LASI				X
India	LASI-DAD	X		X	
Northern Ireland	NICOLA	X		X	
Ireland	TILDA	X	X		

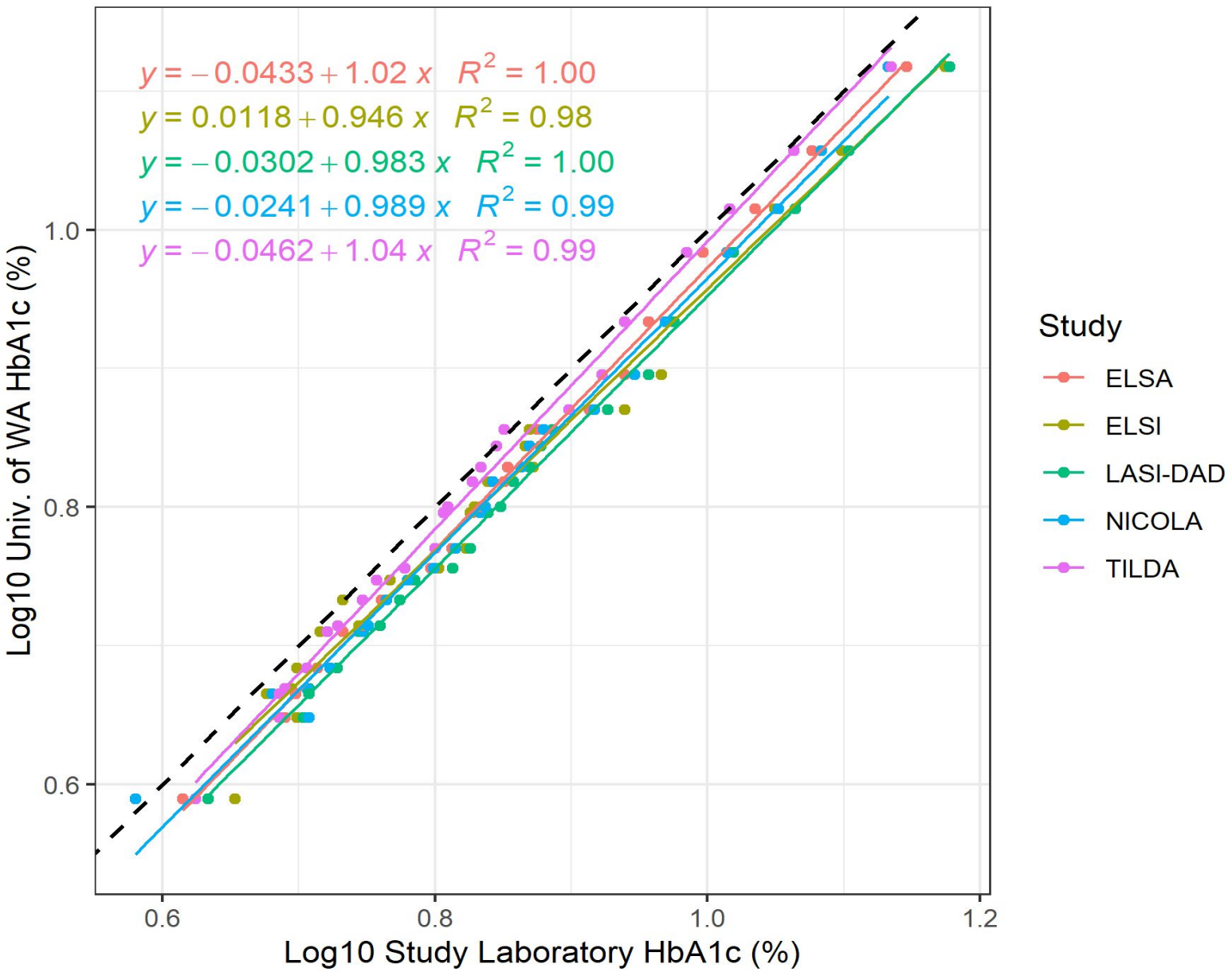
Study Analyzers for HbA1c

Country	Study	HbA1c Analyzer
China	CHARLS	N/A
England	ELSA	TOSOH G8
Brazil	ELSI-Brazil	Bio-Rad Variant Turbo
United States	HRS	N/A
Indonesia	IFLS	Bio-Rad D-10
India	LASI	Roche Cobas 400
India	LASI-DAD	Bio-Rad D-10
Northern Ireland	NICOLA	Werfen ILab 600 (Ireland lab)
Ireland	TILDA	ADAMS A1c HA-8180V

Very high correlations between the biomarker results from all study laboratories and University of Washington:

All correlation coefficients between individual lab values and UW values were 0.99 or 1.00

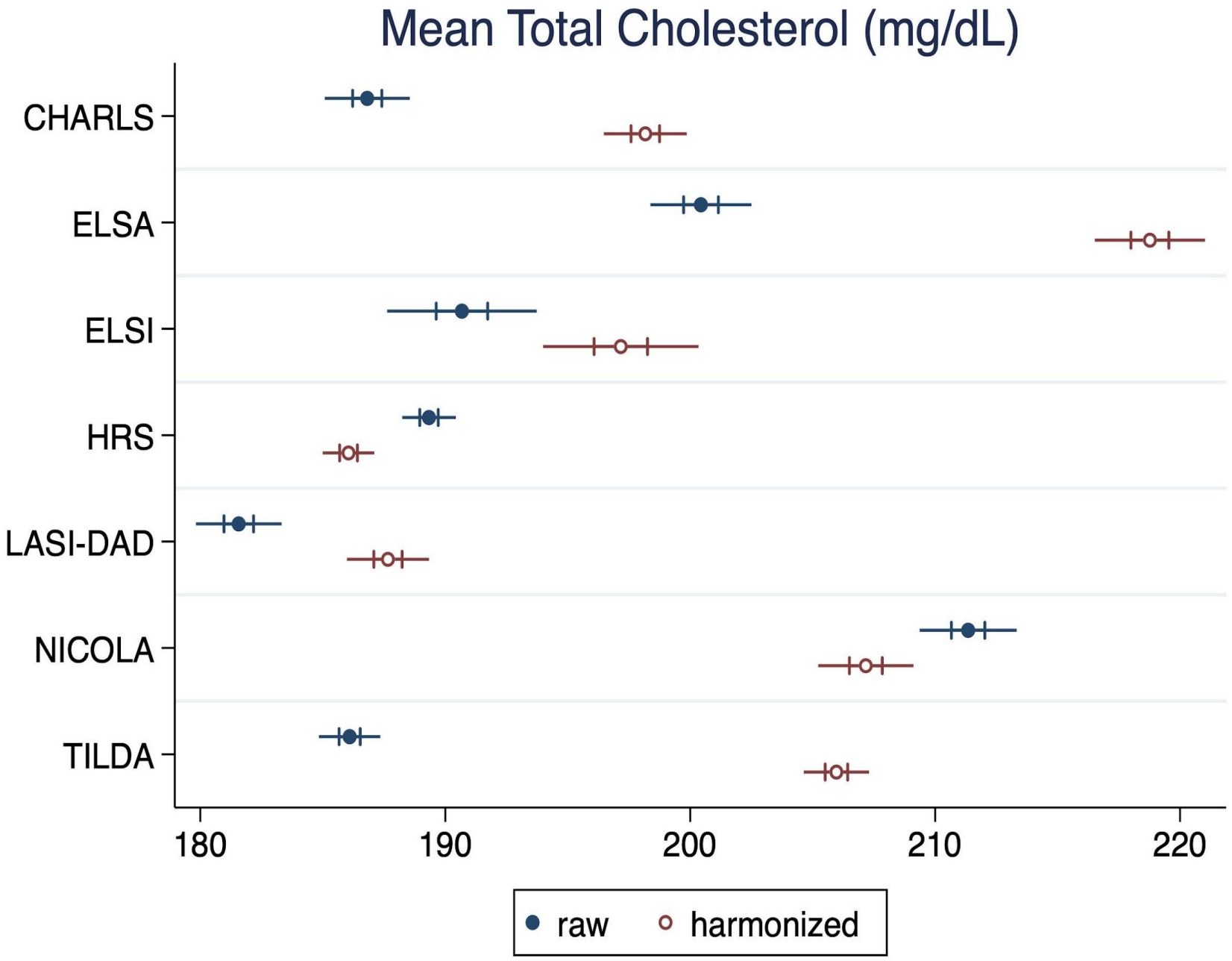
Conclusion: All the laboratories essentially produced the same rank order to the data, which is indicative that all of the laboratories have produced high quality data for their studies.



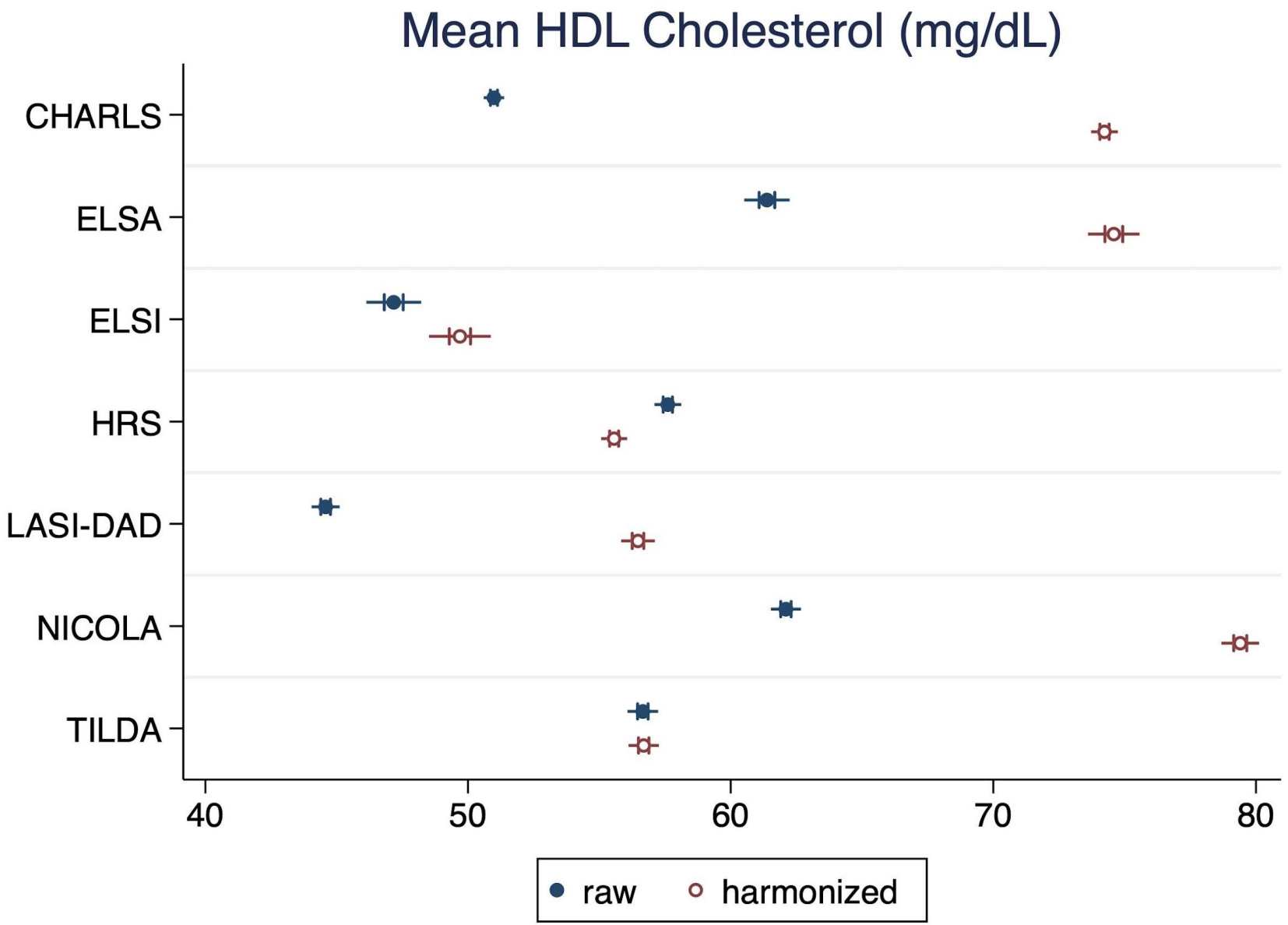
However, the absolute values of the assays differ .

Equations that link HbA1c to the Univ of Washington lab value

Harmonize to get comparable values



Study Population: Raw and Harmonized values for Total Cholesterol



Study Populati
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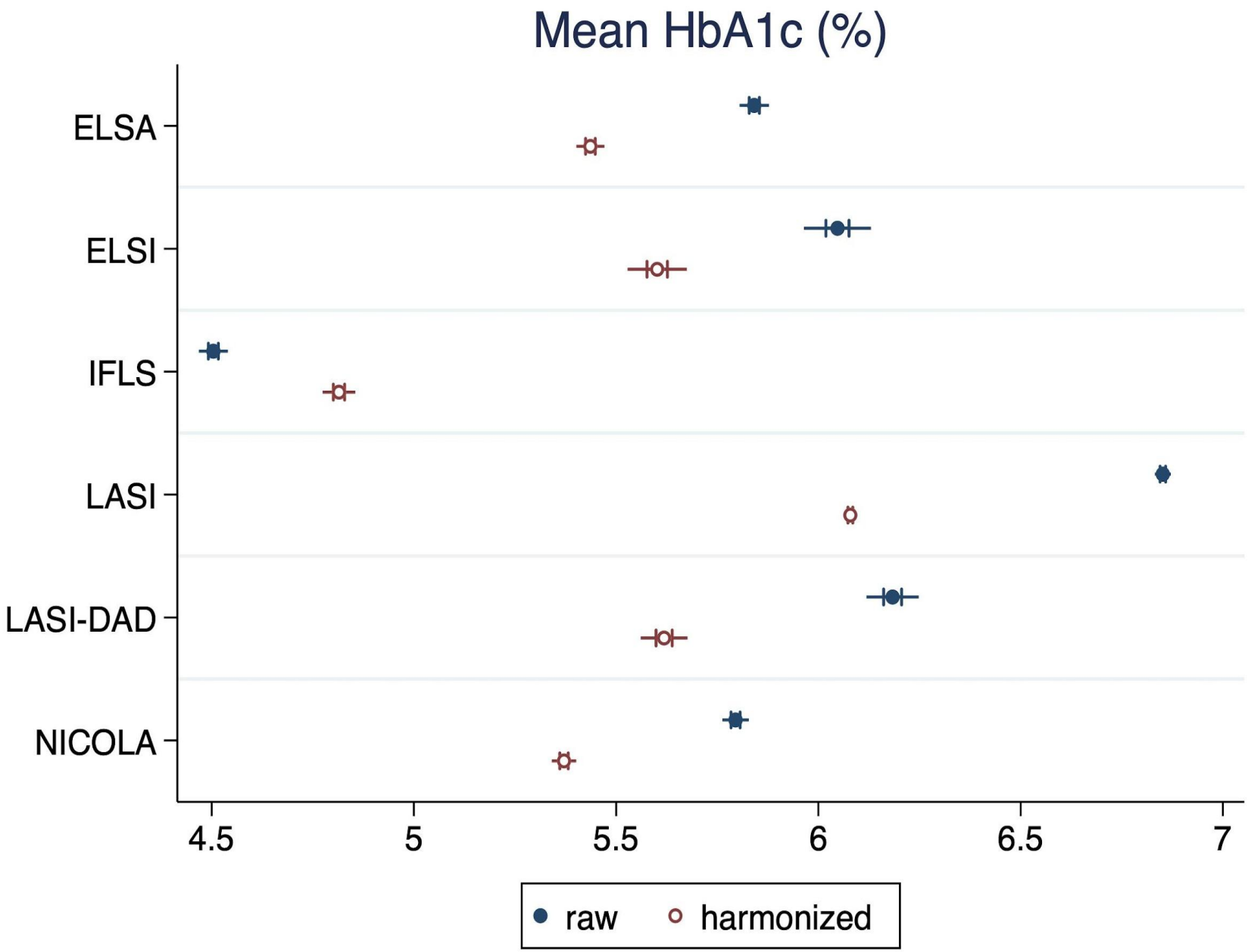
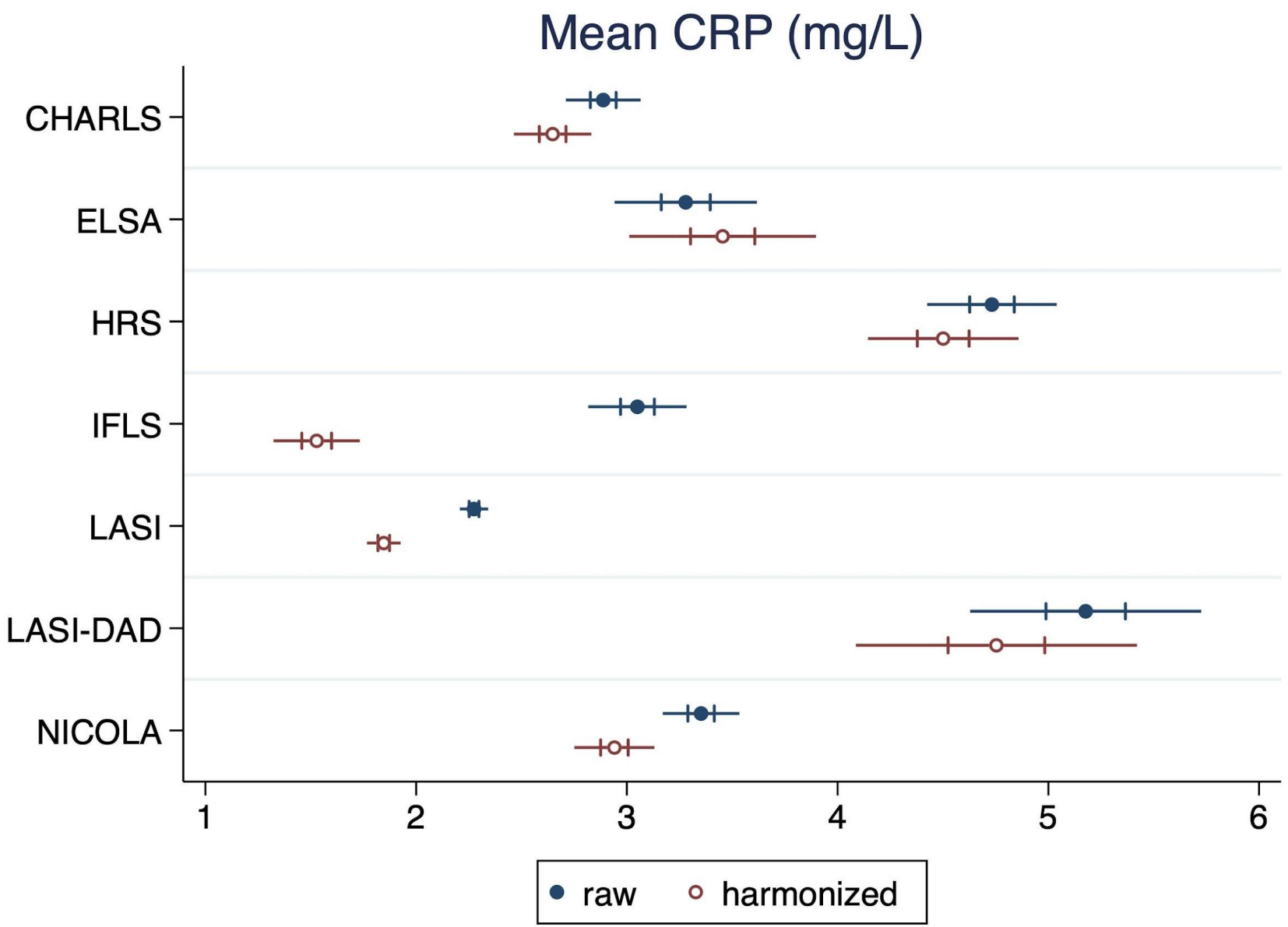
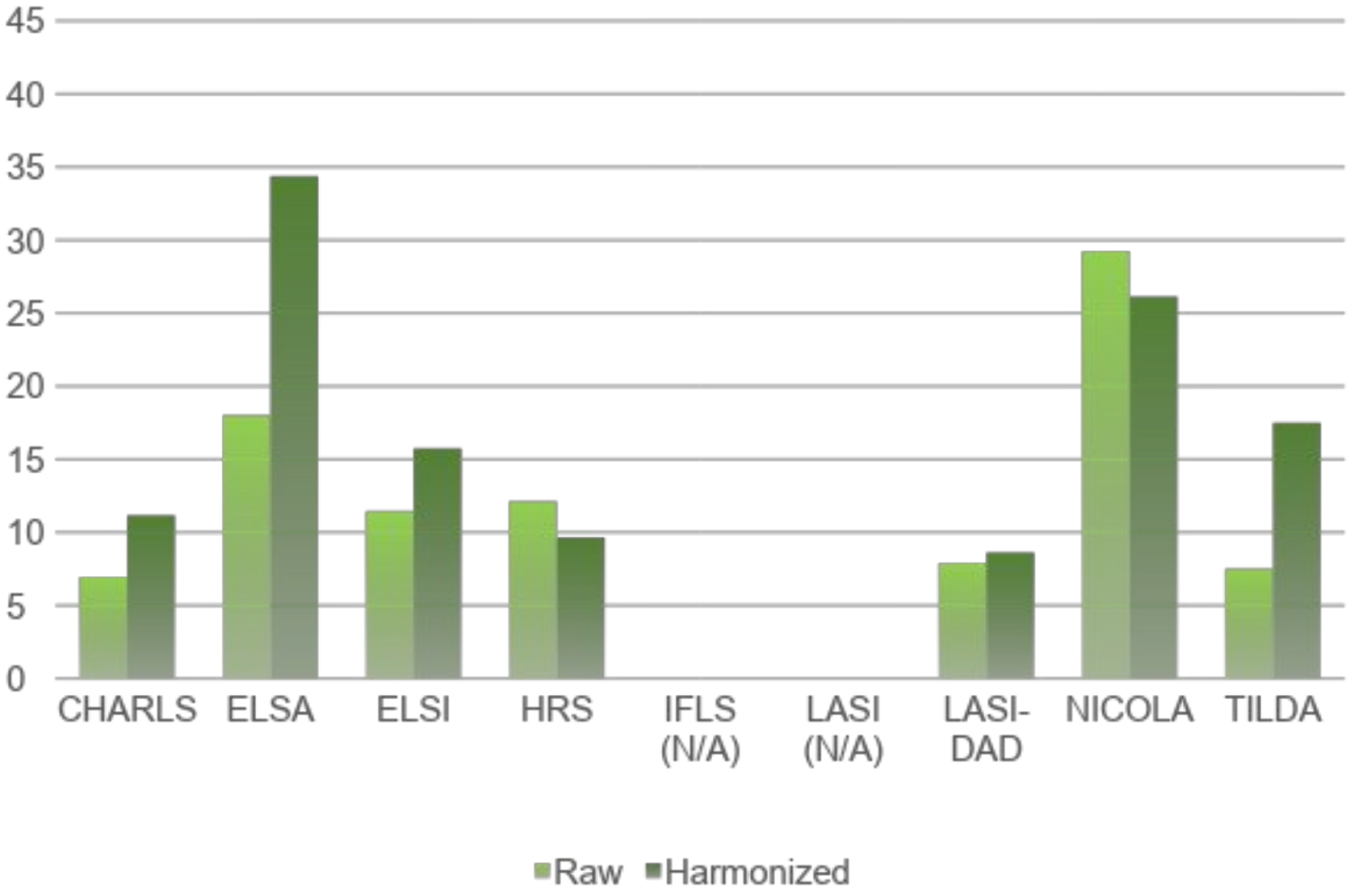


Study Population: Raw and Harmonized values for HbA1c

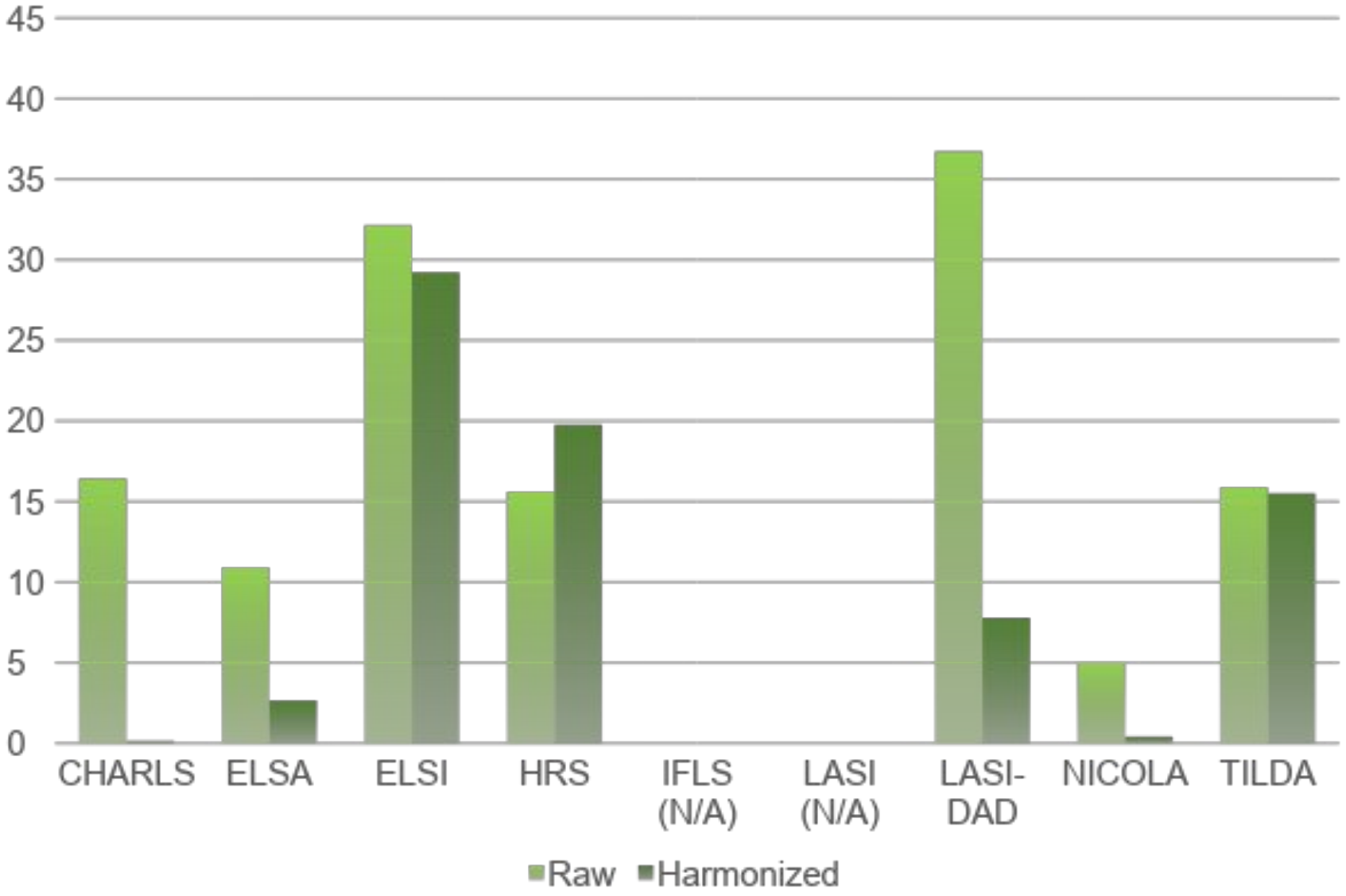


Study Population: Raw and Harmonized values for CRP

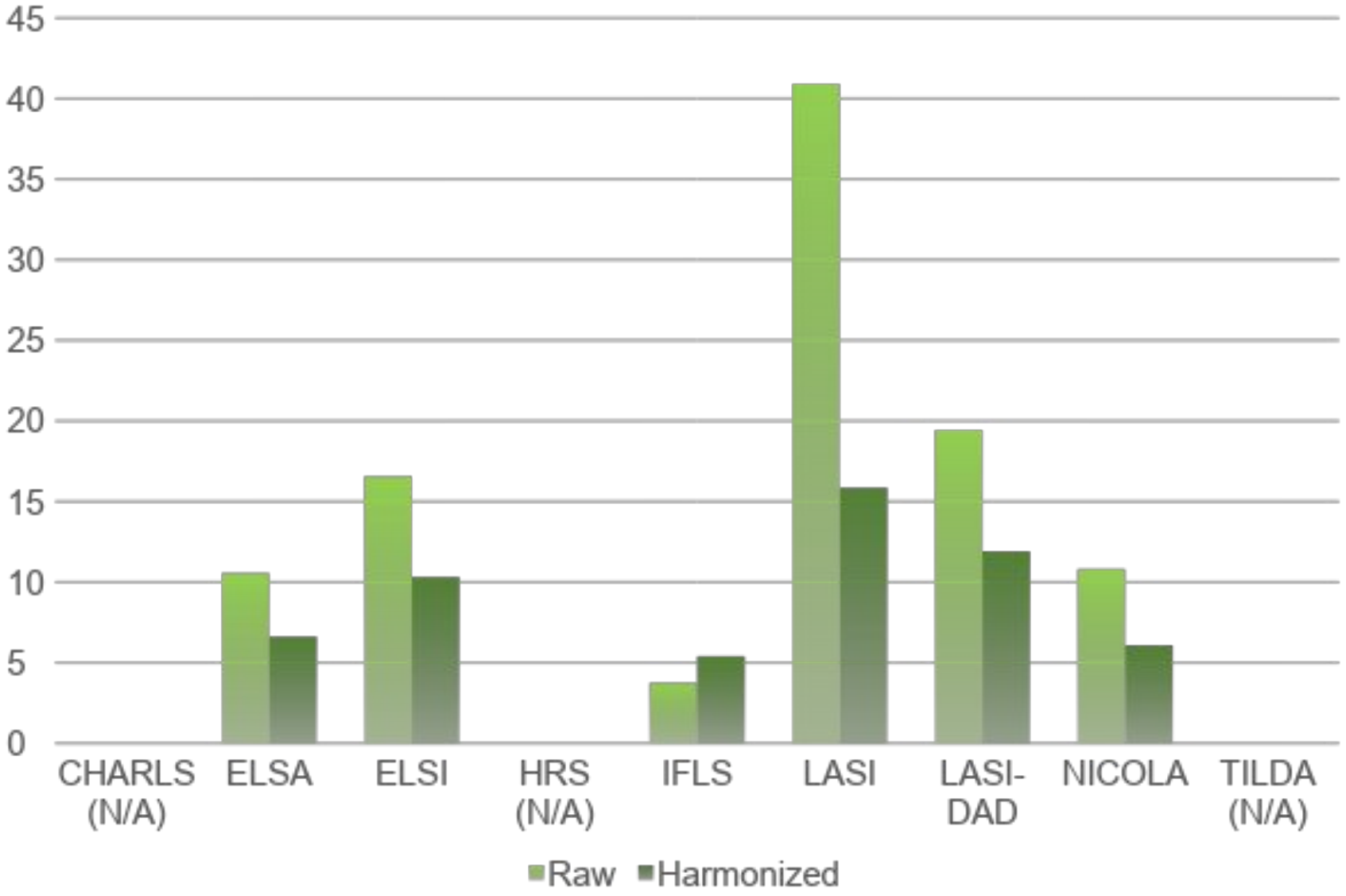
Percent with High-Risk Total Cholesterol: Raw vs Harmonized



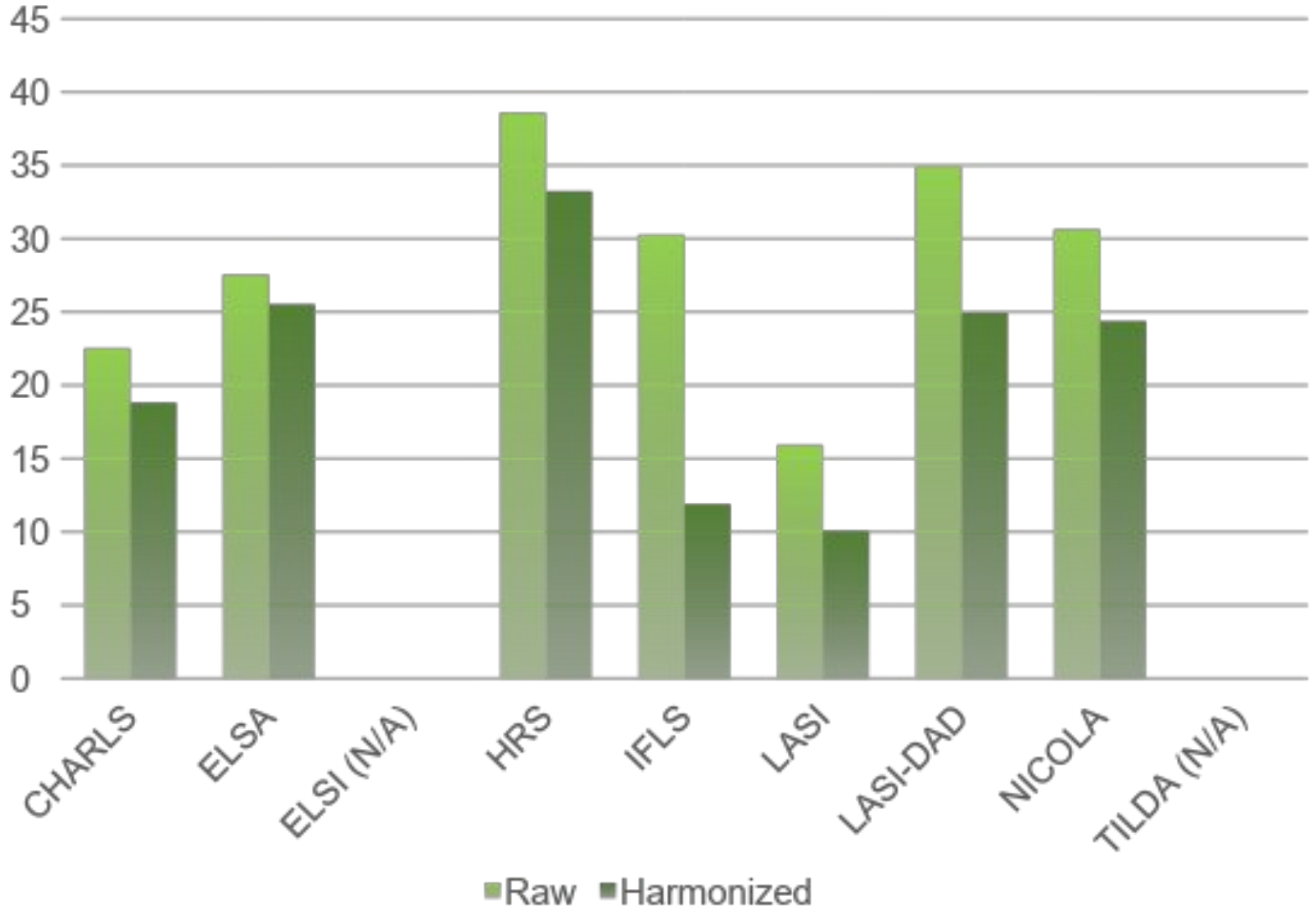
Percent with High-Risk HDL Cholesterol: Raw vs Harmonized



Percent with High-Risk HbA1c: Raw vs Harmonized



Percent with High-Risk CRP: Raw vs Harmonized

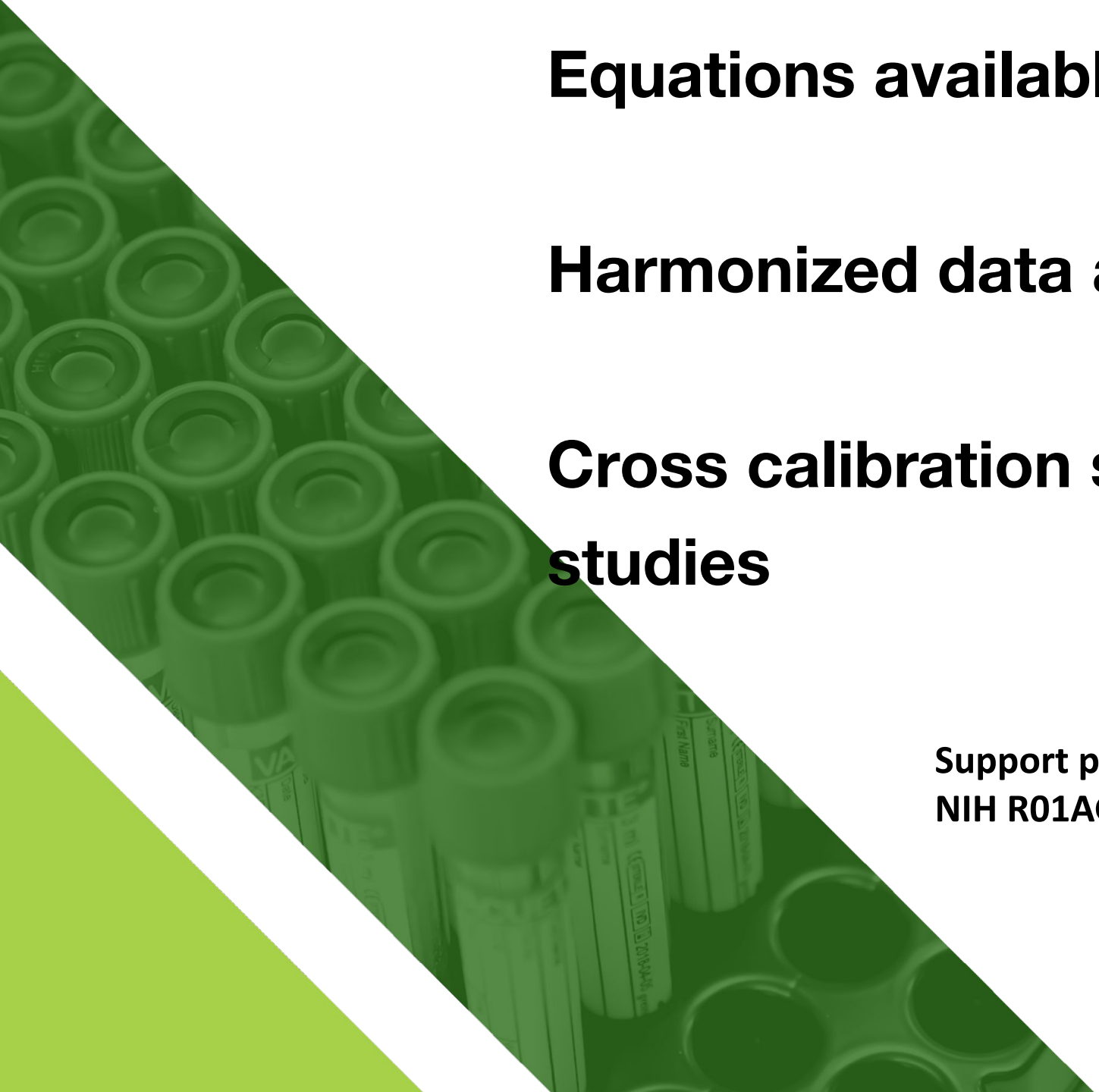


Conclusions

- Labs excellent in producing the relative ranking of respondents – both venous blood and DBS
- Absolute values of assays vary with machines and reagents
- Some assays linked to diagnosis and treatment are less variable across labs
- Comparisons without harmonization can lead to false conclusions

Limitations

- We have only controlled for laboratory conditions



Equations available

Harmonized data available to studies

Cross calibration sample data available to studies

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NICOLA: Frank Kee (PI), Amy Jayne McKnight (PI), Charlotte Neville, Jayne Woodside, Maciej Domanski

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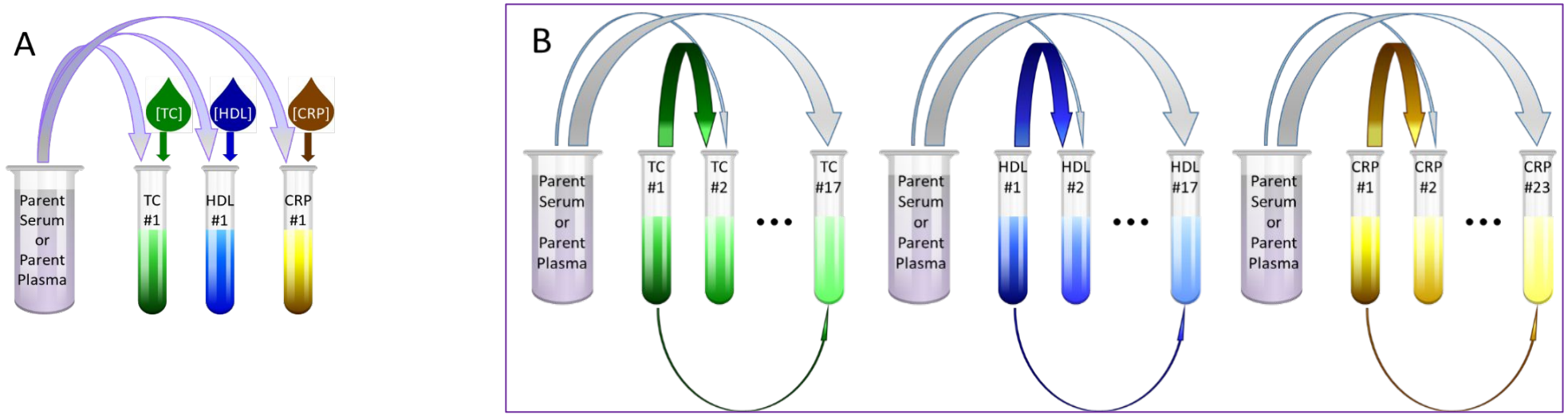
VACUETTE
3 ml
K3E K3EDTA

Protocol – Making Samples for Labs

The distributions of analyte values were obtained by spiking or diluting samples to achieve higher or lower values.

The serum and plasma were chemically stripped of TC, HDL-C and CRP.

To create a set of samples, an aliquot of the serum or plasma parent volume was augmented with an analyte concentrate to produce a sample with a high analyte concentration. Then samples with analyte concentrations spanning a rational physiologic range were created by combining decreasing aliquots of assay validation sample #1 with increasing aliquots of the serum or plasma parent.



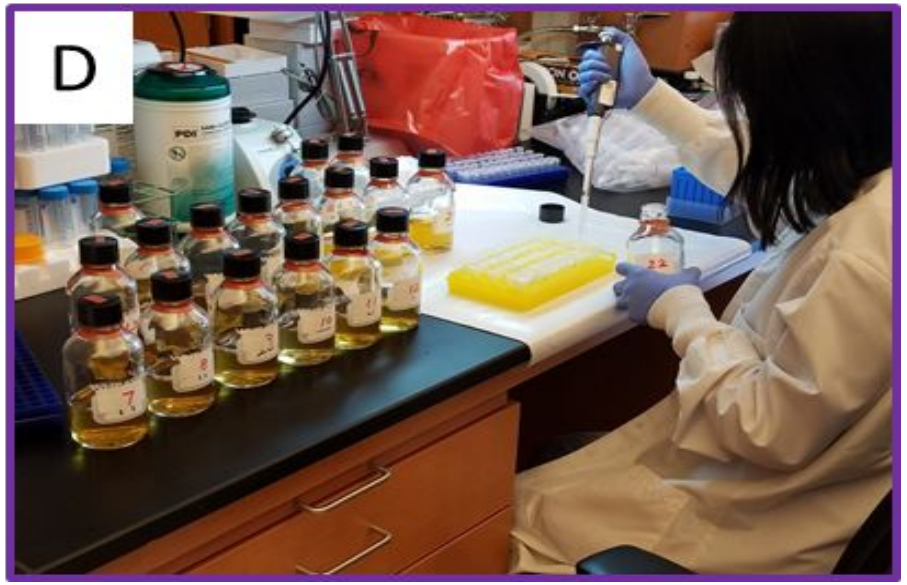
Each daughter subset consists of 24 samples (C).

For TC and HDL-C, there are 17 assay validation samples, and seven assay quality assurance (QA) samples.

For CRP, there are 23 assay validation samples and one assay QA sample.

These samples were analyzed on five consecutive days to establish the analyte concentrations.

1mL aliquots from each of the daughter subsets have been distributed into barcoded vials assigned a random ID number (D) to create 36 replicate sets per analyte for serum (864 samples per subset, 2592 samples total) and 10 replicate sets per analyte for plasma (240 samples per subset, 720 samples total).

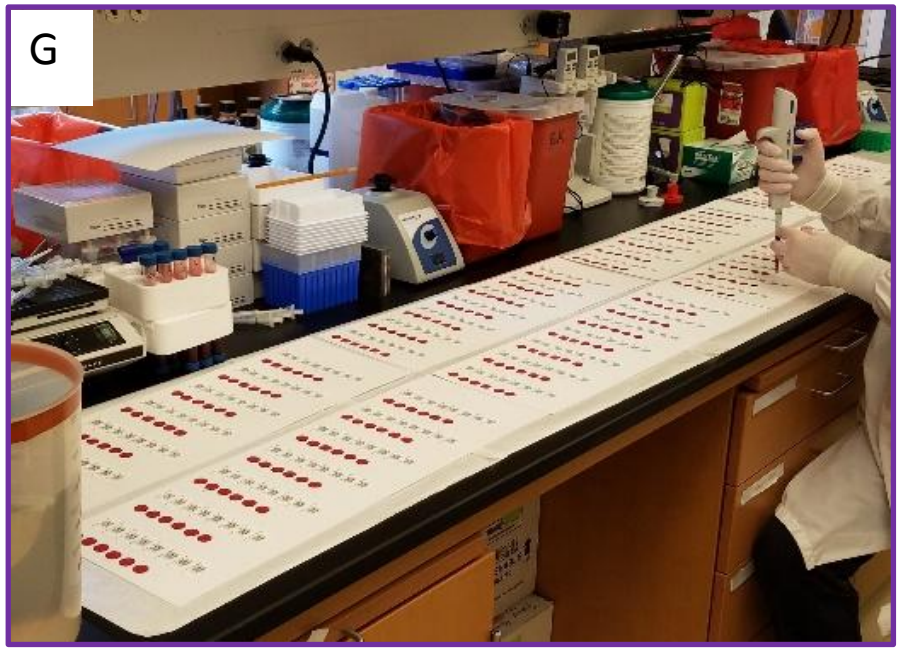


To create the DBS HbA1c samples, 0.075mL aliquots of the HbA1c blood samples have been pipetted onto barcoded Whatman filter paper strips to create 24 DBS samples with HbA1c values matching those of the blood samples (G).

To create the DBS CRP samples, washed red blood cells (wRBC) have been combined with an equal volume from each sample in the plasma CRP subset. 0.075mL aliquots from the plasma CRP-wRBC combinations have been placed onto barcoded filter paper strips to create 24 DBS samples with CRP concentrations matching those of the plasma CRP samples (H).

15 replicate sets (360 DBS total) of the DBS HbA1c samples and of the DBS CRP samples have been created. The DBS samples were analyzed on five consecutive days to establish the %HbA1c values and CRP concentrations.

G



H

Relationships between the UW harmonization samples and samples from each laboratory indicated by equation below:

$$\text{Study Laboratory Harmonization Sample} = \hat{\beta}0 + \hat{\beta}1\text{UW}$$

Subsequently, the coefficients from these equations for each laboratory applied to the logged raw data from the study population to estimate harmonized versions of the study data:

$$\text{Harmonized Study Value} = (\text{Study Raw Value} - \hat{\beta}0) / \hat{\beta}1$$

Characteristics of Study Population Harmonized for this Analysis

Study	Age Eligible (Years)	Sample Size	Year of Collection
CHARLS	50+	8,510	2015
ELSA	50+	2,710	2016-2017
ELSI	50+	2,361	2015-2016
HRS	56+	9,189	2016
IFLS	50+	2,600	2014-2015
LASI	50+	46,517	2017-2019
LASI-DAD	60+	2,892	2017-2019
NICOLA	50+	3,424	2017
TILDA	53+	4,829	2014-2015
TOTAL		83,032	