Recalibration of biochemistry measurements in a multinational cohort study: LIFE course study in CARdiovascular disease Epidemiology (LIFECARE)

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ABSTRACT

Background: Various cardiovascular biomarkers are used to assess and compare the risk of cardiovascular diseases across populations. However, artefactual variations due to the use of different laboratories may make these comparisons invalid. This work describes the inter-laboratory variations in a multi-country cohort, LIFECARE, and the use of recalibration to a reference laboratory to minimise this variability.

Methods: LIFECARE is a cohort of 10,479 participants recruited from Indonesia, Malaysia, Philippines and Thailand between 2008 and 2011, with blood samples analysed at country-specific laboratories (n=4). Thailand was the designated reference laboratory. The measurements from each laboratory were compared against the reference laboratory using a common set of samples analysed at all laboratories, using the MethComp package in R. Laboratory values for cohort participants were recalibrated using the equation generated by the package, if large, statistically significant differences were observed during the comparison.

Results: Glucose, total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride measurements were reported for all four countries. Cholesterol and HDL from all laboratories required recalibration while glucose did not. Recalibration altered the proportions of the population at risk substantially, with prevalence of high cholesterol changing from
56.3% to 75.0% in Malaysia, 52.1% to 37.5% in Indonesia and 31.3% to 22.7% in Philippines. Prevalence of low HDL was similarly altered.

**Conclusion:** There was significant variation in serum lipid levels measured by different laboratories, leading to variations in estimates of population at risk. Recalibration to a reference laboratory can overcome this variability and facilitate meaningful comparisons of laboratory data across countries.

**Keywords:** recalibration, analytical variation, laboratory variation, biomarkers, cardiovascular diseases, Asia
INTRODUCTION

Cardiovascular disease (CVD) is the single most important cause of death globally, with 32% of all deaths, and 46% of non-communicable disease (NCD) deaths being attributed it in 2013[1,2]. Of the estimated 17 million CVD deaths in 2013, 8 million were due to ischemic heart disease, and 6 million due to stroke [3]. The growing burden of CVD has been driven mainly by the demographic and epidemiological transitions in Asia, which has seen the biggest jumps in CVD deaths between 1990 and 2013, with an increase of 97% in South Asia and 47% in East Asia[3].

The risk factors for ischaemic heart disease and stroke are well known and have been identified consistently across countries and regions. However, there are substantial variations in the prevalence of these risk factors, both within and across countries, as well as across studies [4]. In 2011, low income countries showed the lowest, and upper-middle-income countries the highest, prevalence of diabetes, whereas the prevalence of
elevated total cholesterol was highest in Europe, followed by the Americas, Africa and South-East Asia, respectively [5].

These variations in the prevalence of risk factors may be real - due to different lifestyles, socioeconomic status, ethnic groups and genetic predisposition; but may also be artefactual - due to differences in methods of estimation. This artefactual variation is of importance as prevention strategies and interventions are based on assumptions of population risk, and such variation can lead to an erroneous estimation of risk and consequently an inappropriate allocation of prevention efforts and resources. The use of different assays, instruments, analytic and calibration reagents can introduce systematic errors in the measurement of biochemical parameters. Such variability and bias have been reported for a variety of biochemical analytes, including glucose and lipids [6-9].

Analytical variability interferes with the meaningful comparison of data on risk across studies, or even within studies where multiple laboratories are involved. This is especially true of cross-country epidemiological studies, with large numbers of participants spread across multiple countries and the use of multiple laboratories [10]. Hence there is need to devise effective means to make data from different laboratories comparable. In this paper, we demonstrate the use of recalibration to a reference laboratory to minimise variability in the estimation of common cardiovascular risk biomarkers, namely fasting glucose and lipids, across laboratories, using data from a multinational cohort study. We also
demonstrate the effect of laboratory variations on population risk estimates, when using raw results.

**METHODS**

**Recalibration study**

Convenience sampling of 63 participants (male=34, female=29) aged above 21 years was done from an outpatient clinic in a local hospital in Singapore. Ethics approval was obtained from National Health Care Group Domain Specific Review Board. Written, informed consent was obtained from each participant. Blood samples were obtained from all participants after an overnight fast. We excluded samples with a high degree of haemolysis. A total of 57 fresh frozen plasma samples and 54 serum samples were shipped to local laboratories in four countries, including the reference laboratory in Division of Clinical Chemistry, Faculty of Medicine, Mahidol University. This reference laboratory is ISO 15189 certified and is a participating laboratory for the CDC lipid standardization program, with performance within the acceptable criteria of the National Cholesterol Education Program [11,12]. All laboratories measured glucose and lipids (total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides) on these samples. The results obtained from these samples were used to generate the recalibration equations as described in the section on data analysis below.

**LIFECARE study**
LIFECARE is a multinational cohort study conducted in four South East Asian countries (Thailand, Malaysia, Philippines, and Indonesia) [13]. The aim of the LIFECARE study is to identify factors that underlie the changes in cardiovascular risk factors over time in these four countries and determine the impact of changes in these risk factor levels on Health-Related Quality of Life and health services utilization. It was initiated in 2008 and the baseline study was completed in 2011.

Indonesia: 3502 participants from urban and semi-urban areas in Makassar, Indonesia were recruited between 2009 and 2011. Ethics approval was obtained from the Review Board of the Faculty of Medicine, Hasanuddin, University of Makassar.

Malaysia: 2533 participants were recruited between 2010 and 2011 from urban and suburban regions of Sarawak. Ethics approval was obtained from the Medical Research & Ethical Committee, Ministry of Health, Malaysia.

Philippines: 3078 participants were recruited from urban and rural areas in Metro Manila and four nearby provinces between 2009 and 2011[14]. Ethics approval was obtained from the University of the Philippines Manila Research Ethics Board and the Ethics Review Board of the Cardinal Santos Medical Centre.

Thailand: The study was conducted from May 2008 to November 2009. The participants were from the Electricity Generating Authority of Thailand (EGAT) study, a longitudinal study comprising of three waves of recruitment, referred to as EGAT 1, 2, and 3. The participants included in the LIFECARE study were from the third EGAT 2 survey in 2008 (n =
2,286) and the first EGAT 3 survey in 2009 (n = 2,584) [15,16]. Ethics approval was obtained from the Institutional Review Board at Mahidol University.

Consent

Written informed consent was obtained from each participant before the start of the study.

Exclusion

We excluded participants who were not within the pre-defined age range of 18-50 years (Indonesia:4, Malaysia:1, The Philippines:6 and Thailand:1533). For Indonesia, we also excluded 1904 participants from the same households, and 56 participants whose questionnaire and laboratory data could not be linked. Hence, the final number of participants included in the analysis was 10,479 (Indonesia:1538, Malaysia:2532, The Philippines:3072 and Thailand:3337).

Measurements

Table 1 shows the details of sample collection and processing in each of the four countries, as well as the coefficients of variation of the individual tests during the study. LDL-C level was directly measured in all countries except Malaysia, where it was calculated using the Friedewald formula.

Definitions
We defined cut-offs of the various biomarkers for identifying proportion of the populations with risk factors. Diabetes mellitus was defined as fasting plasma glucose level of ≥126 mg/dL. High cholesterol was defined as total cholesterol level of ≥ 200mg/dL. Low HDL-C was defined as HDL-C < 40 mg/dL in males and < 50 mg/dL in females. High LDL-C was defined as LDL-C ≥ 130 mg/dl and high triglycerides was defined as levels ≥150mg/dL.

Data analysis

Biomarkers from each country were recalibrated against the measurements made in the reference laboratory, using a workflow (Figure 1) adapted from Bendix Carstensen’s recommendations for the statistical analysis of method comparison studies and the MethComp package in R [17,18].

Each dataset, comprising both original laboratory and reference measurements of a set of samples, was verified to exhibit constant variance before a recalibration equation was estimated. Data with non-constant variance was log-transformed. Alternative transformations such as the square root transformation were applied if the logarithm transformation did not remEDIATE the non-constant variance. The recalibration equation consisted of a slope term and a constant and was estimated using the MethComp package in R as described by Carstensen [17,18].
| Country     | Laboratory                                      | Samples           | Instruments                                      | Sample collection                                                                 | Sample storage                                                                 | Sample processing                                                                                                                                                                                                                                                                                                                                 | Coefficient of variation for the tests                                      |
|-------------|------------------------------------------------|-------------------|--------------------------------------------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Thailand    | Division of Clinical Chemistry, Faculty of Medicine, Mahidol University | serum samples     | Dimension RxL MAX analyser (Siemens Healthcare Diagnostics, Inc.) | All samples were collected after overnight fasting. Samples for glucose measurement were collected in fluoride oxalate tubes. | Samples were kept between 2°C -8°C until process and analysed on the same day. | The plain tubes for lipids were centrifuged for 10 minutes at 3000 rpm using a centrifuging machine (Kokusan H-27F centrifuge).                                                                                              | glucose (1.6), total cholesterol (1.8 ), triglycerides (1.7) HDL (2.4) and LDL (1.95) |
| Indonesia   | Prodia lab, Makassar                            | serum samples     | Cobas c 501 analyser (Roche; German)            | All samples were collected after overnight fasting. Samples for glucose measurement were collected in fluoride oxalate tubes. | Samples were kept between 2°C -8°C until process and analysed on the same day. | The plain tubes for lipids were centrifuged for 10 minutes at 3000rpm using a centrifuging machine (Eppendorf centrifuge 5702)                                                                                              | glucose (2.66), total cholesterol (2.95), triglycerides (4.58), HDL (2.85) and LDL (2.27) |
| Malaysia    | Sarawak General Hospital                       | serum samples     | Olympus AU400 & 640 (CLIAwived.com, San Diego), COBAS Integra 800 (Roche; Germany), Hitachi 912 (Roche; Germany) | All samples were collected after overnight fasting. Samples for glucose measurement were collected in fluoride oxalate tubes. | Samples were kept between 2°C -8°C until process and analysed on the same day. | The plain tubes for lipids were centrifuged for 10 minutes at 4000rpm using a centrifuging machine (Eppendorf Centrifuge 5702)                                                                                              | glucose (3.68), total cholesterol (4.49), triglycerides (4.21) and HDL (6.17) in Malaysia |
| Philippines | Philippine General Hospital, University of     | heparinized plasma samples for lipid | COBAS Integra 400 plus (Roche; German)           | All samples were collected after overnight fasting. Samples for glucose measurement were collected in fluoride oxalate tubes. | Samples were kept between 2°C -8°C until process and analysed on the same day. | The plain tubes for lipids were centrifuged for 5 minutes at 5000 rpm using a centrifuging machine (Eppendorf Centrifuge 5702)                                                                                              | glucose (3.34), total cholesterol (1.20), triglycerides                     |
| Country     | Measurement                      | Description                                                                 | Coefficients of Variation |
|-------------|----------------------------------|-----------------------------------------------------------------------------|----------------------------|
| Philippines | glucose measurement              | same day for samples collected in the area near Manila and kept at <-30°C and analysed within three days for samples collected in the remote areas. | rpm (Hettich Zentrifugen EBA 20) (1.76), HDL (1.60) and LDL (3.72) |

Table 1: Details of sample collection, processing and coefficients of variation for the tests
The decision to apply the recalibration equation was based on an examination of the slope (estimate and significance level) and constant (estimate, limits of agreement) terms for each analyte. If the slope term suggested large, statistically significant differences in measurements between samples, the full equation was applied. If the slope term indicated only small, non-significant differences in measurements between samples, and the limits of agreement (LOA) did not contain zero, the analyte was recalibrated by adding the constant term. All analytes with non-significant slope terms had LOAs that contained zero hence these were not recalibrated.

The recalibrated measurements were summarized by their mean and standard deviation. To compare the difference in biomarker values and prevalence between genders, Mann Whitney U test and Pearson’s chi-squared test were used respectively. To investigate if biomarker values and prevalence differed across 10-year age group categories (< 30, 30-39, 40 and above), the age groups were arranged in ascending order and Cuzick’s test for linear trend was used.

Bland-Altman (BA) plots were used to detect persistent low-quality measurements in the datasets. We first identified samples that deviated from the random scatter about zero in each BA plot for each biomarker. If a sample was identified three or more times across different biomarkers within each country, the quality of the measurement was classified as low. These low-quality measurements were removed and the workflow was reapplied to the amended dataset for a separate recalibration.
RESULTS

Table 2 reports the socio-demographic and medication use characteristics of the participants from Indonesia, Malaysia, Philippines and Thailand. Participants from Indonesia, Malaysia and Philippines were predominantly female, whereas Thailand had the highest proportion of men (71.7%). Almost half the participants from Indonesia and Thailand were above 40 years of age, while Malaysia and Philippines had a roughly equal distribution of ages across the three age groups.

Recalibration with reference laboratory

Glucose, total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride measurements were reported for all four countries. Following the workflow, five country-specific biomarkers were transformed to fulfil the constant variance assumption. 16 out of the 25 five country-specific biomarkers had large statistically differences from reference laboratory measurements and required recalibration with an equation. The remaining nine country-specific biomarkers were not recalibrated.

Variation between biomarkers

Table 3 illustrates the recalibration parameters used on each biomarker from each country. Between biomarkers, it is evident that glucose performed the best, as it did not require recalibration against the reference lab. Cholesterol and HDL most often required recalibration.

Variation between countries

Table 4 reports the original and recalibrated measurements along with the prevalence of different dyslipidaemias in the sample populations based on these measurements. Prevalence of high cholesterol increased
from 56.3% to 75.0% in Malaysia after recalibration but decreased in Indonesia and Philippines (Indonesia: 52.1% to 37.5%; Philippines: 31.3 to 22.7%).

Table 2: Sociodemographic and medication use characteristics in the LIFECARE study

|                  | Indonesia N=1538 | Malaysia N=2532 | Philippines N=3072 | Thailand N=3337 |
|------------------|------------------|-----------------|---------------------|-----------------|
| **Age group**    |                  |                 |                     |                 |
| <30 years        | 227 (14.7%)      | 694 (27.4%)     | 861 (28.0%)         | 200 (6%)        |
| 30 - 39 years    | 491 (6%)         | 967 (1%)        | 110 (3%)            | 841 (25.2%)     |
| >40 years        | 820 (31.9%)      | 871 (38.1%)     | 2 (35.8%)           | 229 (68.8%)     |
|                  | 2 (9)            | 110 (7)         | 7 (6)               |                 |
|                  | 53.3%            | 34.4%           | 36.1%               |                 |
| **Gender**       |                  |                 |                     |                 |
| Male             | 394 (25.6%)      | 105 (41.7%)     | 132 (43.2%)         | 239 (71.7%)     |
| Female           | 114 (7.4%)       | 8 (3%)          | 9 (6%)              | 1 (1%)          |
|                  | 4 (74.3%)        | 147 (58.2%)     | 174 (56.7%)         | 944 (28.2%)     |
|                  | 8 (4)            | 1 (3)           | 4 (9)               |                 |
| **Current Smoker**|                 |                 |                     |                 |
| No               | 135 (87.8%)      | 203 (80.1%)     | 222 (72.3%)         | 268 (80.5%)     |
| Yes              | 1 (4%)           | 7 (3%)          | 6 (9%)              | 1 (8%)          |
| Missing          | 175 (11.3%)      | 502 (19.8%)     | 849 (27.6%)         | 618 (18.5%)     |
|                  | 12 (8)           | 0 (3)           | 4 (30)              | 2 (2)           |
|                  | 0.78             | 0               | 0                   | 0.9             |
| **Alcohol consumed in past year** |                  |                 |                     |                 |
| No               | 160 (63.5%)      | 126 (41.0%)     | 126 (37.7%)         |                 |
| Yes              | 923 (36.4%)      | 181 (58.9%)     | 201 (60.3%)         |                 |
| Missing          | 0 (5)            | 2 (8)           | 3 (2)               |                 |
|                  | 0                | 0               | 63 (1.89)           |                 |
| **On DM medication** |                 |                 |                     |                 |
| No               | 147 (96.0%)      | 250 (98.8%)     | 303 (98.7%)         | 325 (97.5%)     |
| Yes              | 7 (4%)           | 5 (2%)          | 3 (3%)              | 3 (6%)          |
| Missing          | 31 (2.02)        | 29 (11.5%)      | 39 (12.7%)          | 18 (0.54)       |
|                  | 30 (1.95)        | 0               | 0                   | 63 (1.89)       |
| **On hypertension medication** |                 |                 |                     |                 |
| No               | 961 (62.4%)      | 239 (94.7%)     | 296 (96.4%)         | 295 (88.5%)     |
| Yes              | 45 (8%)          | 8 (5%)          | 8 (5%)              | 5 (5%)          |
| Missing          | 532 (2.93)       | 133 (5.25)      | 108 (3.52)          | 269 (8.06)      |
|                  | 34.5 (1.04)      | 0               | 0                   | 113 (3.39)      |
| **On high cholesterol medication** |                 |                 |                     |                 |
| No               | 146 (94.9%)      | 245 (97)        | 305 (99.5)          | 289 (86.7)      |
| Yes              | 1 (9)            | 6 (2.76)        | 8 (4)               | 4 (3)           |
| Missing          | 48 (3.12)        | 70 (2.4)        | 14 (0.46)           | 199 (5.96)      |
|                  | 29 (1.89)        | 6               | 0                   | 244 (7.31)      |
| Country | Biomarker | Scale of measurement | N | LOA   | Slope | p-value for Slope | Constant |
|---------|-----------|----------------------|---|--------|--------|-------------------|----------|
| **Full data** | Malaysia | Glucose* | Linear | 57 | -8.120 to 7.937 | 1.00 | 0.993 | -0.072 |
| Cholesterol | Linear | 54 | -44.063 to 86.869 | 0.72 | 0.009 | 68.152 |
| HDL | Linear | 54 | -20.479 to 18.462 | 0.72 | <0.00 | 14.642 |
| LDL* | Linear | 54 | -18.273 to 64.816 | 0.88 | 0.212 | 34.163 |
| TG | Log | 54 | -0.217 to 0.554 | 0.84 | 0.001 | 0.821 |
| **Indonesia** | Glucose* | Linear | 57 | -4.058 to 11.847 | 1.05 | 0.067 | -1.265 |
| Cholesterol | Linear | 54 | -65.404 to 43.589 | 0.70 | <0.00 | 50.267 |
| HDL | Linear | 54 | -24.606 to 7.865 | 0.81 | 0.010 | 3.818 |
| LDL | Linear | 54 | -59.644 to 22.459 | 0.68 | <0.00 | 1 |
| TG* | Log | 54 | -0.213 to 0.253 | 0.95 | 0.131 | 0.238 |
| **Philippines** | Glucose* | Linear | 57 | -5.710 to 9.856 | 1.01 | 0.537 | 0.376 |
| Cholesterol | Linear | 54 | -23.965 to 0.083 | 0.93 | 0.005 | 0.736 |
| HDL | Log | 54 | -0.124 to 0.015 | 1.04 | 0.017 | -0.250 |
| LDL | Log | 54 | -0.062 to 0.092 | 0.95 | 0.036 | 0.209 |
| TG | Linear | 54 | -10.711 to 6.711 | 0.97 | 0.014 | 0.967 |
| **Low quality measurements removed** | Malaysia | Glucose* | Linear | 52 | -8.128 to 7.937 | 1.00 | 0.993 | -0.072 |
| Cholesterol | Linear | 49 | -40.785 to 71.865 | 0.75 | 0.010 | 58.260 |
| HDL | Linear | 49 | -19.936 to 14.767 | 0.77 | 0.002 | 11.042 |
| LDL* | Linear | 49 | -17.036 to 56.801 | 0.89 | 0.178 | 30.752 |
| TG | Square root | 49 | -0.774 to 1.849 | 0.94 | 0.139 | 1.053 |
| **Indonesia** | Glucose* | Linear | 50 | -1.209 to 9.889 | 1.04 | 0.060 | 0.637 |
| Cholesterol | Linear | 47 | -49.713 to 40.861 | 0.78 | 0.014 | 36.772 |
| HDL | Linear | 47 | -21.323 to 7.749 | 0.83 | 0.020 | 3.982 |
| LDL | Linear | 47 | -46.664 to 19.557 | 0.74 | <0.00 | 19.973 |
| TG* | Log | 47 | -0.176 to 0.259 | 0.97 | 0.553 | 0.132 |

*: These analytes had p-value > 0.05 when checking for constant difference suggesting no requirement for calibration
The same trend was observed after removing low quality measurements for Malaysia and Indonesia. A pronounced increase in prevalence of low HDL from 36.2% to 61.2% was observed in Indonesia, with moderate changes in Malaysia and Philippines (Malaysia: 22.5% to 15.0%, Philippines: 56.9% to 66.1%). A similar pronounced decrease in prevalence of high LDL was also evident in Indonesia (55.5% to 28.2%).

In general, the trends in prevalence corresponded with the trends in mean values and ranges of the biomarkers. In Malaysia, mean recalibrated HDL values were lower than the original values, yet the prevalence of low HDL decreased due to a reduction in the range after recalibration. Changes in prevalence of lipid abnormalities persisted after removing low quality measurements.

**Variation across gender and age categories**

On stratifying the data by gender, it was observed that females, in general, had a significantly more favourable profile than males for diabetes, high cholesterol, and high triglycerides (Table 5). The difference was most pronounced in Malaysia. The significant differences in prevalence between the two genders persisted after recalibration. Prevalence of high cholesterol (HC) and high TG (HT) in Malaysia increased and remained
# Table 4: Comparison of calibrated and uncalibrated lipid profile measurements among countries

|                | Cholesterol | HDL | LDL | TG | Glucose |
|----------------|-------------|-----|-----|----|---------|
|                | Mean ± SD   | High Cholesterol (≥200mg/dL) | Mean ± SD (mg/dL) | Low HDL (< 40 mg/dL in male, < 50 mg/dL in female) | Mean ± SD (mg/dL) | High LDL (≥130mg/dl) | Mean ± SD (mg/dL) | High TG (≥150mg/dL) | Mean ± SD (mg/dL) | DM (≥126mg/dL) |
| **Malaysia**   |             |     |     |    |         |
| No calibration | 208.21 ± 40.37 | 1424 | 56.28 | 55.46 ± 14.39 | 569 | 22.4 | 127.11 ± 39.04 | 1130 | 45.0 | 121.54 ± 81.72 | 603 | 23.84 | 88.57 ± 19.45 | 65 | 2.57 |
| Calibrated     | 219.52 ± 29.35 | 1898 | 75.02 | 55.07 ± 10.49 | 380 | 15.0 | 130.16 ± 71.49 | 690 | 27.28 |
| Calibrated*    | 215.87 ± 30.56 | 1756 | 69.41 | 53.86 ± 11.11 | 499 | 1 | 130.16 ± 71.49 | 690 | 27.28 |
| **Indonesia**  |             |     |     |    |         |
| No calibration | 204.38 ± 41.35 | 801 | 52.08 | 51.14 ± 11.38 | 557 | 36.2 | 135.60 ± 36.18 | 853 | 55.5 | 135.93 ± 91.98 | 479 | 31.14 | 92.67 ± 36.28 | 92 | 6.08 |
| Calibrated     | 193.33 ± 28.94 | 577 | 37.52 | 45.39 ± 9.25 | 941 | 61.1 | 117.93 ± 24.78 | 495 | 28.1 |
| Calibrated*    | 197.82 ± 32.58 | 664 | 43.17 | 46.53 ± 9.47 | 872 | 8 | 120.73 ± 26.88 | 495 | 28.1 |
| **Philippines**|             |     |     |    |         |
| No calibration | 184.28 ± 42.14 | 961 | 31.28 | 44.81 ± 13.18 | 1749 | 56.9 | 113.67 ± 36.83 | 929 | 30.2 | 127.32 ± 77.88 | 819 | 26.66 | 98.89 ± 43.18 | 159 | 5.18 |
| Calibrated     | 173.59 ± 39.53 | 696 | 22.66 | 41.97 ± 12.98 | 2030 | 66.0 | 115.14 ± 35.81 | 969 | 31.5 |

#: Low quality measurements removed
| Gender | Cholesterol | HDL | LDL | TG |
|--------|-------------|-----|-----|----|
|        | Mean ± SD (mg/dL) | High Cholesterol (≥200 mg/dL) | Mean ± SD (mg/dL) | Low HDL (< 40 mg/dL in male, < 50 mg/dL in female) | Mean ± SD (mg/dL) | High LDL (≥130 mg/dL) | Mean ± SD (mg/dL) | High TG (≥150 mg/dL) |
|        | N | % | N | % | N | % | N | % |

**Malaysia**

|          | Males | Females |          | Males | Females |          | Males | Females |          | Males | Females |          |
|----------|-------|---------|----------|-------|---------|----------|-------|---------|----------|-------|---------|----------|
| No       | 214.24 ± 41.55* | 203.89 ± 38.95 | 217.00 ± 352 | 60.21 ± 60.21 | 329.00 ± 352 | 188.00 ± 217 |
| calibration | 671 | 753 | 20.51* | 38.95 | 133.75 ± 39.15 | 53.09* | 38.95 | 151.26 ± 95.94 |
|          | 255 | 305 | 23.88 | 122.45 ± 122.45 | 39.4 | 100.27 ± 100.27 |
|          | 217 | 305 | 23.88 | 122.45 ± 122.45 | 39.4 | 100.27 ± 100.27 |
| Calibrated | 223.90 ± 30.21* | 216.38 ± 28.31 | 217.00 ± 352 | 60.21 ± 60.21 | 329.00 ± 352 | 188.00 ± 217 |
| Calibrated | 835 | 1063 | 79.07* | 8.07* | 50.25 ± 50.25 | 7.56* | 156.94 ± 81.96 |
|          | 790 | 966 | 74.81* | 8.54* | 48.75 ± 48.75 | 12.67* | 111.00 ± 55.53 |
|          | 220.44 ± 31.45* | 212.60 ± 29.48 | 217.00 ± 352 | 60.21 ± 60.21 | 329.00 ± 352 | 188.00 ± 217 |
|          | 305 | 361 | 52.03 | 112 | 28.43* | 135.68 ± 135.68 | 215 | 54.71 | 169.48 ± 107.11 |
|          | 444 | 444 | 52.03 | 112 | 28.43* | 135.68 ± 135.68 | 215 | 54.71 | 169.48 ± 107.11 |
| Indonesia | No | Males | 204.97 ± 31.45* | 205 | 52.03 | 44.69 ± 9.07* | 112 | 28.43* | 135.68 ± 135.68 | 215 | 54.71 | 169.48 ± 107.11 |
|          | Females | 47.02 ± 65.54 | 596 | 52.10 | 9.07* | 444 | 38.90 | 39.82 | 638 | 55.77 | 107.11* |
|          | 278 | 278 | 51.02* | 278 | 51.02* | 278 | 51.02* | 278 | 51.02* | 278 | 51.02* | 278 | 51.02* | 278 | 51.02* |
Table 5: Comparison of lipid biomarkers with respect to gender among countries

*: Significantly different across genders (p<0.05)
#: Low quality measurements removed

| Country     | Calibrated | Calibrated* | Philippines | No calibration | Calibrated |
|-------------|------------|-------------|-------------|----------------|------------|
|             | Males      | Females     | Males       | Females        | Males      |
|             | 204.17 ±   | 39.22       | 193.75 ±    | 32.92          | 198.29 ±   |
|             | 152        | 38.58       | 152         | 425            | 166        |
|             | 40.15 ±    | 11.26       | 40.15 ±     | 7.37*          | 41.16 ±    |
|             | 204        | 737         | 204         | 737            | 183        |
|             | 51.78*     | 64.42       | 51.78*      | 64.42          | 46.45*     |
|             | 117.98 ±   | 27.27       | 117.98 ±    | 27.27          | 120.78 ±   |
|             | 109        | 234         | 117.91 ±    | 324            | 128        |
|             | 27.74      | 28.32       |             |                | 32.57      |
|             |            |             |             |                |            |
| Philippines |            |             |             |                |            |
| No calibration |            |             |             |                |            |
| Males       | 183.92 ±   | 411         | 184.56 ±    | 550            | 173.25 ±   |
|             | 30.93      | 31.55       | 30.93       | 31.55          | 23.10      |
|             | 41.97 ±    | 12.31*      | 41.97 ±     | 12.31*         | 39.18 ±    |
|             | 640        | 1109        | 640         | 1109           | 789        |
|             | 111.76 ±   | 48.16*      | 111.76 ±    | 48.16*         | 113.27 ±   |
|             | 367        | 562         | 367         | 562            | 388        |
|             | 27.61*     | 32.24       | 27.61*      | 32.24          | 29.19*     |
|             | 150.87 ±   | 93.29*      | 150.87 ±    | 93.29*         | 147.31 ±   |
|             | 505        | 314         | 505         | 314            | 484        |
|             |            |             |             |                | 36.42*     |
| Females     | 44.58      | 550         | 46.99 ±     | 31.55          | 40.19      |
|             | 12.31*     | 1120        | 12.31*      | 1120           | 13.40      |
|             | 48.16*     | 63.63       | 48.16*      | 63.63          | 36.61      |
|             | 115.12 ±   | 109.37 ±    | 115.12 ±    | 109.37 ±       | 147.31 ±   |
|             | 109.37 ±   | 562         | 109.37 ±    | 562            | 484        |
|             |            | 32.24       | 18.01       |                |            |
|             |            |             |             |                |            |
| Calibrated  |            |             |             |                |            |
| Males       | 183.92 ±   | 411         | 184.56 ±    | 550            | 173.25 ±   |
|             | 23.10      | 23.10       | 23.10       | 23.10          | 307        |
|             | 39.18 ±    | 12.12*      | 39.18 ±     | 12.12*         | 39.18 ±    |
|             | 789        | 1241        | 789         | 1241           | 789        |
|             | 113.27 ±   | 59.37*      | 113.27 ±    | 59.37*         | 113.27 ±   |
|             | 388        | 581         | 388         | 581            | 388        |
|             | 29.19*     | 33.33       | 29.19*      | 33.33          | 29.19*     |
|             | 147.31 ±   | 90.49*      | 147.31 ±    | 90.49*         | 147.31 ±   |
|             | 484        | 287         | 484         | 287            | 484        |
|             |            | 16.47       | 16.47       |                |            |
| Females     | 44.11 ±    | 71.20       | 44.11 ±     | 71.20          | 44.11 ±    |
|             | 71.20      | 1241        | 71.20       | 1241           | 71.20      |
|             | 116.56 ±   | 59.37*      | 116.56 ±    | 59.37*         | 116.56 ±   |
|             | 381        | 581         | 381         | 581            | 381        |
|             | 30.19      | 33.33       | 30.19       | 33.33          | 30.19      |
|             | 107.06 ±   | 90.49*      | 107.06 ±    | 90.49*         | 107.06 ±   |
|             | 581        | 287         | 581         | 287            | 581        |
|             |            | 16.47       | 16.47       |                |            |

Table 5: Comparison of lipid biomarkers with respect to gender among countries
significantly different between the genders after recalibration. For Philippines, prevalence of high TG decreased slightly and remained significantly different between males and females after recalibration. The prevalence of low HDL was significantly lower in males across all countries. Prevalence increased for Indonesia and Philippines (Indonesia males: 28.4% to 51.8%, females 38.9% to 64.4%; Philippines males: 48.2% to 59.4%, females 63.6% to 71.2%), but dipped for Malaysia, especially the males (males: 20.5% to 7.6%, females: 23.9% to 20.4%) when using recalibrated data. The extent of change in recalibrated prevalence was attenuated towards the original proportions after removing low quality measurements. In general, the same phenomenon was observed for mean values of the biochemistry variables.

Table 6 shows that the youngest subjects generally had the most favourable profile for high total cholesterol, high LDL and high TG, and the linear trend of increasing prevalence with increasing age was statistically significant. However, the prevalence of low HDL had a significant positive association with age for Malaysia only. Similar linear trends persisted for the above-mentioned profiles after recalibration, with and without removal of low-quality measurements. Large changes in prevalence were observed for all three countries in high cholesterol and low HDL after recalibration. In general, the same phenomenon was observed for mean values of the biomarkers.
| Age group | Cholesterol | HDL | LDL | TG |
|-----------|-------------|-----|-----|----|
|           | Mean ± SD (mg/dL) | High Cholesterol (≥200mg/dL) | Mean ± SD (mg/dL) | Low HDL (< 40 mg/dL in male, < 50 mg/dL in female) | Mean ± SD (mg/dL) | High LDL (≥130mg/dL) | Mean ± SD (mg/dL) | High TG (≥150mg/dL) |
|           | years | N | % | N | % | N | % | N | % |
| **Malaysia** | | | | | | | | | |
| No calibration | <30 | 197.19 ± 37.64* | 313 | 45.10 | 56.67 ± 14.31* | 123 | 17.72 | 119.07 ± 36.44* | 243 | 35.22 |
| | 30-40 | 210.04 ± 40.18 | 556 | 57.56 | 55.02 ± 14.42 | 221 | 23.27 | 127.99 ± 39.16 | 441 | 46.08 |
| | 40-50 | 214.96 ± 40.91 | 555 | 63.79 | 54.99 ± 14.37 | 221 | 25.37 | 132.99 ± 39.07 | 446 | 51.80 |
| Calibrated | <30 | 211.51 ± 27.36* | 450 | 64.84 | 55.95 ± 10.43 | 82 | 11.62 | 111.95 ± 58.16* | 285 | 18.40 |
| | 30-40 | 217.26 ± 29.21 | 749 | 77.54 | 54.75 ± 10.51 | 143 | 16.03 | 135.16 ± 73.08 | 278 | 29.53 |
| | 40-50 | 224.43 ± 29.74 | 699 | 80.34 | 54.73 ± 10.48 | 143 | 16.03 | 139.15 ± 76.64 | 314 | 31.95 |
| Calibrated* | <30 | 211.51 ± 27.36* | 400 | 57.64 | 54.79 ± 11.04 | 104 | 14.99 | 111.95 ± 58.16* | 285 | 18.40 |
| | 30-40 | 217.26 ± 29.21 | 698 | 72.26 | 53.52 ± 11.13 | 200 | 26.68 | 135.16 ± 73.08 | 278 | 29.53 |
| | 40-50 | 224.43 ± 29.74 | 658 | 75.63 | 53.49 ± 11.10 | 143 | 16.03 | 139.15 ± 76.64 | 314 | 31.95 |
| **Indonesia** | | | | | | | | | |
| No calibration | <30 | 179.82 ± 32.23* | 53 | 23.35 | 52.30 ± 11.13 | 71 | 31.28 | 115.71 ± 29.33* | 67 | 29.52 |
| | 30-40 | 198.47 ± 42.32 | 230 | 46.84 | 51.25 ± 11.72 | 197 | 50.12 | 132.17 ± 37.26 | 535 | 51.12 |
| | 40-50 | 214.71 ± 39.43 | 518 | 63.17 | 51.25 ± 11.72 | 289 | 51.24 | 143.18 ± 34.85 | 535 | 65.32 |
| Calibrated | <30 | 176.14 ± 22.56* | 35 | 15.42 | 46.33 ± 9.05 | 122 | 53.74 | 104.30 ± 25.52 | 112 | 27.81 |
| | 30-40 | 189.20 ± 29.63 | 152 | 30.96 | 44.80 ± 9.53 | 309 | 62.93 | 115.57 ± 25.52 | 294 | 22.81 |
| | 40-50 | 200.56 ± 27.60 | 390 | 47.56 | 43.49 ± 8.13 | 510 | 62.20 | 123.12 ± 34.85 | 390 | 35.90 |
| Calibrated* | <30 | 178.47 ± 25.40* | 40 | 17.62 | 47.49 ± 9.26 | 110 | 48.46 | 105.95 ± 21.79* | 31 | 13.66 |
| | 30-40 | 193.17 ± 33.35 | 180 | 36.66 | 45.92 ± 9.75 | 296 | 60.29 | 118.17 ± 27.68 | 334 | 26.48 |
| | 40-50 | 205.96 ± 31.07 | 444 | 54.15 | 46.63 ± 9.34 | 466 | 56.83 | 126.35 ± 25.89 | 466 | 40.78 |
| Philippines |  <30 | 30-40 | 40-50 |  <30 | 30-40 | 40-50 |
|-------------|------|-------|-------|------|-------|-------|
| No calibration | 170.49 ± 38.46* | 44.81 ± 12.52 | 54.59 | 102.20 ± 32.32* | 57.53 | 113.76 ± 64.64* |
| 30-40 | 185.06 ± 39.92 | 44.76 ± 13.16 | 58.16 | 113.86 ± 36.72 | 44.88 ± 13.70 | 126.29 ± 73.17 |
| 40-50 | 194.22 ± 44.08 | 44.76 ± 13.16 | 58.16 | 122.38 ± 37.83 | 44.88 ± 13.70 | 138.88 ± 89.27 |
| Calibrated | 160.65 ± 36.07* | 40.04 | 30.12 | 103.99 ± 31.55* | 66.97 | 113.32 ± 62.70* |
| 30-40 | 174.32 ± 37.44 | 41.97 ± 12.32 | 53.30 | 115.33 ± 35.70 | 67.36 | 123.47 ± 70.98 |
| 40-50 | 182.92 ± 41.35 | 41.92 ± 12.97 | 54.76 | 123.61 ± 36.69 | 73.60 | 135.68 ± 86.60 |

Table 6: Comparison of lipid biomarkers with respect to age among countries
*
#: Low quality measurements removed
CONCLUSIONS

In our study, we found that there was substantial variation among the measurements from different laboratories, especially for lipids. This variability could result either from the pre-analytic stage (i.e. procedure in the field) or from the analytic stage (i.e. laboratory assessment). At the pre-analytic stage, sample collection, storage, processing and transportation may introduce variability. All study sites followed the same procedures for sample collection. Philippines analysed lipids from plasma samples whereas the other countries analysed them from serum, which may lead to differences in the measured concentrations, though of small magnitude [19,20]. Due to the distance from the study site to the laboratory, the Philippine team had to centrifuge and separate plasma before transporting the samples to the laboratory for analysis. However, storage at different temperatures might not be a source of major variability as the samples were analysed within a few days and bacterial contamination was avoided[19,20]. Hence, the significant variability between laboratories observed in our study is likely to have occurred at the analytic stage.

Variation at analytic stage can result from methods of determination (enzymatic or chemical methods), the calibrator, instruments and the reagents used. In the LIFECARE study, all the laboratories used enzymatic methods, so the variability can be attributed to the differences in calibrators, instruments or the reagents. In our study, we found that there was a
significant change in the values of almost all lipid components after recalibration. The change was most prominent in the prevalence of high LDL, which decreased from 55.5% to 32.2% after recalibration, and the prevalence of low HDL, which increased from 36.2% to 56.7% in Indonesia. One of the reasons that LDL and HDL are more prone to variability than other analytes could be the heterogeneity of LDL and HDL in terms of particle size, density, shape, lipid and apolipoprotein composition, with different assays measuring different subclasses of particles [21,22].

Significantly greater magnitude of bias and total errors have been reported while estimating lipid levels in individuals with disease, compared those without disease [21]. In addition, abnormally high or low TG levels [23,24], and bias and errors in HDL measurement [25,26] can lead to under- and over-estimation of LDL-C levels by the Friedewald formula.

As seen from the results of our study, the magnitude and direction of error for a biomarker can vary between laboratories and even between different biomarkers measured within the same lab. In Indonesia, for instance, people were being over-diagnosed with high LDL and underdiagnosed for low HDL within the same laboratory based on the original measurements. Such variability has important clinical implications. Diagnosis and treatment of cardiovascular risk factors like diabetes and dyslipidaemias is based on cut-offs recommended by national and international guidelines, and not individual laboratory reference ranges. LDL-C is the primary target
for medical treatment of hypercholesterolemia due to proven efficacy in CVD risk reduction [27], and an overestimation can result in CVD risk misclassification into high risk. This might result in unnecessary treatment of a patient whose LDL-C levels can be managed just by dietary control and physical activity. Conversely, underestimation can result in misclassification of a person who actually has high risk into a low risk category resulting in a failure to treat and reduce risk appropriately. In terms of population health, this variability in estimates may lead to erroneous projections of population risk and disease burden, leading to inaccurate prioritization of the issue and allocation of resources disproportionate to the need.

Hence, it is crucial to standardize the measurements of these analytes in order to have a meaningful comparison when we study the cardiovascular risk factors across countries and even within the same country over time. The ideal way to achieve this is by accreditation of laboratories and/or manufacturers for accuracy with a reference gold-standard procedure, such as the lipid standardization programme run by the Centres for Diseases Control in the US [11,28-30]. Such accuracy-based standardization ensures that measurements from any laboratory or any combination of instruments, reagents and assays are directly traceable to the reference measurement [31]. However, most of national and international laboratory certification and external quality assurance programmes compare individual laboratory performance for a specific analyte with pooled means derived from all the laboratories that participate in the programme. While such comparisons can
give an estimate of deviation of laboratory performance against peers, they do not give any information on the accuracy of the results obtained [6,32]. In addition, many of these programmes use lyophilised sera, which may have significant matrix effects, leading to erroneous conclusions about system performance [32,33]. While there are on-going efforts to harmonize quality assurance performance using commutable materials, i.e. materials without matrix effects, and with a focus on traceability to reference standards, this will take time given the sheer number of laboratories around the world, and impetus from national agencies to adopt such targets [34,35].

In the short term, another alternative for epidemiological studies is to recalibrate the results from the various laboratories involved to a reference laboratory, as we have demonstrated in this paper. This may be more feasible when studies are conducted over geographically dispersed populations, where sample storage and transport to a reference laboratory may be more challenging. This reduces pre-analytical variations due to storage conditions and duration. Using an accredited laboratory with a strong quality assurance program for recalibration allows us to improve the accuracy and precision of the biochemical measurements without the need for individual laboratories to invest in new quality assurance initiatives that may be time consuming and economically challenging.

In summary, we have demonstrated artefactual variations in serum lipid levels and prevalence of lipid abnormalities due to variation in
estimation of these parameters between laboratories and showcased a method for recalibration to ensure comparability of the results. Researchers, policy makers and health professionals need to take such artefactual variations into consideration while comparing biochemical data across studies and countries.

**Declarations**

**Ethics approval and consent to participate**

Ethics approvals were obtained from Review Board of Faculty of Medicine, Hasanuddin, University Makassar for Indonesia, Medical Research & Ethical Committee, Ministry of Health, Malaysia for Malaysia, the University of the Philippines Manila Research Ethics Board and the Ethics Review Board of the Cardinal Santos Medical Centre for Philippines, and the Institutional Review Board at Mahidol University for Thailand. Written informed consent was obtained from each participant before the start of the study.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Competing interests**
The authors declare that they have no competing interests.

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**Authors' contributions**

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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