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Calibration of High-Density Lipoprotein Cholesterol Values From the Korea National Health and Nutrition Examination Survey Data, 2008 to 2015

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Background: For correct interpretation of the high-density lipoprotein cholesterol (HDL-C) data from the Korea National Health and Nutrition Examination Survey (KNHANES), the values should be comparable to reference values. We aimed to suggest a way to calibrate KNHANES HDL-C data from 2008 to 2015 to the Centers for Disease Control and Prevention (CDC) reference method values.

Methods: We derived three calibration equations based on comparisons between the HDL-C values of the KNHANES laboratory and the CDC reference method values in 2009, 2012, and 2015 using commutable frozen serum samples. The selection of calibration equation for correcting KNHANES HDL-C in each year was determined by the accuracy-based external quality assurance results of the KNHANES laboratory.

Results: Significant positive biases of HDL-C values were observed in all years (2.85-9.40%). We created the following calibration equations: standard HDL-C = 0.872 × [original KNHANES HDL-C] + 2.460 for 2008, 2009, and 2010; standard HDL-C = 0.952 × [original KNHANES HDL-C] + 1.096 for 2012, 2013, and 2014; and standard HDL-C = 1.01 × [original KNHANES HDL-C] - 3.172 for 2011 and 2015. We calibrated the biases of KNHANES HDL-C data using the calibration equations.

Conclusions: Since the KNHANES HDL-C values (2008-2015) showed substantial positive biases compared with the CDC reference method values, we suggested using calibration equations to correct KNHANES data from these years. Since the necessity for correcting the biases depends on the characteristics of research topics, each researcher should determine whether to calibrate KNHANES HDL-C data or not for each study.

Key Words: HDL cholesterol, Korea National Health and Nutrition Examination Survey, Calibration

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INTRODUCTION

The Korea National Health and Nutrition Examination Survey (KNHANES) comprises a series of studies designed to assess health and nutritional status in the Korean population. KNHANES is one of the most important sources of data for evaluating trends in hyperlipidemia. High-density lipoprotein cholesterol (HDL-C) is a useful marker for evaluating dyslipidemia and therefore was

included in the KNHANES as a routine laboratory test. Accurate HDL-C values are essential for the correct use of the KNHANES data and for generating estimates of hypoalphalipoproteinemia burden in the Korean population. HDL-C data are currently available from the KNHANES, but bias in the direct HDL-C assays has limited the utility of these data. Including HDL-C assays by the KNHANES, most commercially available HDL-assays for medical laboratories use homogenous reagents for direct measurement of HDL-C, facilitating automation and improved imprecision over the previously employed precipitation-based HDL-C methods. However, in a recent study, five of the eight examined direct HDL-C assays met the established goals (total error of ≤13% and bias of ≤5%) of the National Cholesterol Education Program (NCEP) working group in samples from normolipidemic individuals, but all assays failed to meet the desired criteria in samples from patients with cardiovascular disease and/or dyslipidemia [1].

The National Health and Nutrition Examination Surveys (NHANES), which determines the prevalence of chronic disease and health conditions in the general noninstitutionalized civilian US population, suggested using calibration equations for correction of the original serum creatinine values in NHANES 1988-1994 and 1999-2000 to estimate kidney function [2]. Calibration equations were derived from comparing serum creatinine values in NHANES data with standard creatinine values measured using an assay traceable to a known gold-standard reference method [2]. For the cystatin C values in the NHANES 1988-1994 and 1999-2002 results, using equations to convert to ERM471/IFCC-traceable cystatin C has been suggested for data users to ensure harmonization [3].

In this study, we aimed to calibrate the original 2008-2015 KNHANES HDL-C values to the Centers for Disease Control and Prevention (CDC) reference method values, using calibration equations based on comparison studies between the HDL-C values obtained by the KNHANES laboratory and those of the CDC reference method [4, 5].

METHODS

1. KNHANES HDL-C assay

From 2008 to 2015, the Seegene Medical Foundation (Seoul, Korea) analyzed all samples for HDL-C testing for the KNHANES using a Hitachi Automatic Analyzer 7600 (Hitachi Co., Tokyo, Japan) and Cholestest N HDL reagent (Sekisui Medical Co., Tokyo, Japan). HDL-C was measured according to the manufacturer's instructions and standard laboratory procedures. Routine internal and external quality control programs, including the Accuracy-Based Lipids (ABL) Survey of the College of American Pathologists (CAP) and the Lipid Standardization Program (LSP) of the CDC (Atlanta, GA, USA), were performed to monitor accuracy and precision.

2. HDL-C comparison studies

We performed three comparison studies (in September 2009, June 2012, and June 2015) using commutable frozen serum (CFS) samples with a wide range of HDL-C values. The CFS samples were prepared and validated according to the CLSI guideline protocol (C37-A: Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedure; Approved Guideline) [6]. We used 33 samples in 2009, 19 samples in 2012, and 26 samples in 2015 for the comparison studies. All samples were analyzed in duplicate or triplicate during each run of the study, and one run was conducted per day over two days for each comparison study. The HDL-C CDC reference method values of CFS samples in 2009 were determined by the gas chromatography-isotopic dilution mass spectrometric (IDMS) method at the Lipid Reference Laboratory, Clinical Chemistry Branch, CDC, and those in 2012 and 2015 were measured by the Abell-Kendall (AK) method at the CEQAL Inc. (Vancouver, Canada) of the Cholesterol Reference Method Laboratory Network (CRMLN). The IDMS and AK methods are traceable to the CDC reference method (ultracentrifugation/spectrophotometry method for HDL-C in blood serum) [4, 5]. We compared the HDL-C values quantified by the reference measurement procedure to values measured during the KNHANES (uncalibrated HDL-C values) using Passing-Bablok regression for each comparison study [7].

3. Selection of appropriate calibration equation for each year using accuracy-based external quality assurance data

We determined the appropriate calibration equation to apply for each year based on results of accuracy-based external quality assurance (EQA) programs at the Seegene Medical Foundation, which included the LSP (third quarter 2010 through second quarter 2015) and the ABL Survey (2008 through 2012). We performed a trend analysis of routine HDL-C results to determine the need for data correction with a calibration equation. This analysis was performed according to the year of the sample.

4. Statistical analysis

Statistical analyses were performed by using Excel (Microsoft, Redmond, WA, USA) and Analyse-it (Analyse-it Software Ltd.,

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Leeds, United Kingdom). In the calibration studies, we compared mean HDL-C values using a paired Student's t-test. We used Passing-Bablok regression analysis to obtain the calibration equations [7]. All reported *P* values were based on 2-sided tests; *P* values less than 0.05 were considered significant.

RESULTS

Fig. 1 depicts the outline of the present study.

1. KNHANES HDL-C comparison studies

Overall, there was good agreement between the original HDL-C values and reference measurements. Summary statistics and calibration regression equations are presented in Table 1. We derived the calibration regression equations by comparing the uncalibrated HDL-C results with the CDC reference method values using CFS samples. Overall, the mean uncalibrated HDL-C values were higher than the CDC reference method values ($P<0.05$, all). The degrees of overestimation of uncalibrated HDL-C compared with the CDC reference method values were 4.88 mg/dL in 2009, 1.62 mg/dL in 2012, and 3.13 mg/dL in 2015 (Fig. 2). We examined the intercept and slope obtained by Passing-Bablok regression analyses for each calibration equation [7].

2. Selection of the appropriate calibration equation based on accuracy-based EQA data

We determined the need for HDL-C result correction and selected the appropriate calibration equation for each year through trend analysis of EQA program results (Table 2). The number of samples for each year ranged from 6 to 54. ABL Survey results were derived from a single measurement, and LSP results were the mean values of duplicate measurements. Overall agreement between the original HDL-C values and reference measurements was high. However, since significant positive biases ranging from 2.85% to 9.40% were observed in samples obtained in 2008 to 2015, the correction of the HDL-C values was needed for data

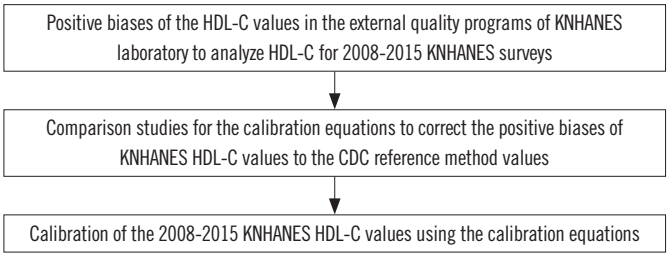

Fig. 1. Flow diagram for the study. Since positive biases ranging from 2.85% to 9.40% were observed in the external quality programs of KNHANES laboratory to analyze HDL-C for 2008-2015 KNHANES surveys, correction of the KNHANES HDL-C values from these years was necessary. Three calibration equations were derived by the comparison studies, which compared the HDL-C values at the KNHANES laboratory with those at the CRMLN laboratory in 2009, 2012, and 2015. The application of the calibration equations to the KNHANES HDL-C values in each year was determined on the basis of the external quality assurance results in the KNHANES laboratory.
Abbreviations: HDL-C, high-density lipoprotein cholesterol; CDC, Centers for Disease Control and Prevention; KNHANES, Korea National Health and Nutrition Examination Surveys; CRMLN, Cholesterol Reference Method Laboratory Network.

Table 1. Summary statistics of the comparison studies for the calibration equations

	2009 Comparison study (N = 33)	2012 Comparison study (N = 19)	2015 Comparison study (N = 26)
Mean uncalibrated HDL-C (SD)	55.81 (6.31)	56.24 (10.05)	58.61 (10.95)
Mean CDC reference HDL-C values (SD)	50.92 (5.62)	54.77 (9.71)	55.48 (11.39)
Mean HDL-C difference (uncalibrated value - reference value) (95% CI)	4.88 (4.33 to 5.44)	1.62 (0.64 to 2.61)	3.13 (2.35 to 3.91)
*P**	<0.001	0.0029	<0.001
Passing-Bablok regression†			
Intercept (95% CI)	-2.82 (-15.90 to 3.05)	-1.15 (-8.04 to 3.78)	3.14 (0.27 to 8.58)
Slope (95% CI)	1.15 (1.03 to 1.41)	1.05 (0.95 to 1.19)	0.99 (0.89 to 1.05)
Calibration equations‡			
Intercept	2.460	1.096	-3.172
Slope	0.872	0.952	1.01

*Paired t-test of the hypothesis that the means are equal between uncalibrated HDL-C and CDC reference method values (mg/dL); †Passing-Bablok regression was done by uncalibrated HDL-C values as dependent variables (Y) and CDC reference method values as independent variables (X); ‡Calibration equations were derived by exchanging of the variables of Passing-Bablok regression (CDC reference method values as independent variables and uncalibrated HDL-C as dependent variables) for the correction of uncalibrated HDL-C values of KNHANES laboratory to the CDC reference method values.
Abbreviations: HDL-C, high-density lipoprotein cholesterol; CDC, Center for Disease Control and Prevention; CRMLN, Cholesterol Reference Method Laboratory Network; CI, confidence interval.

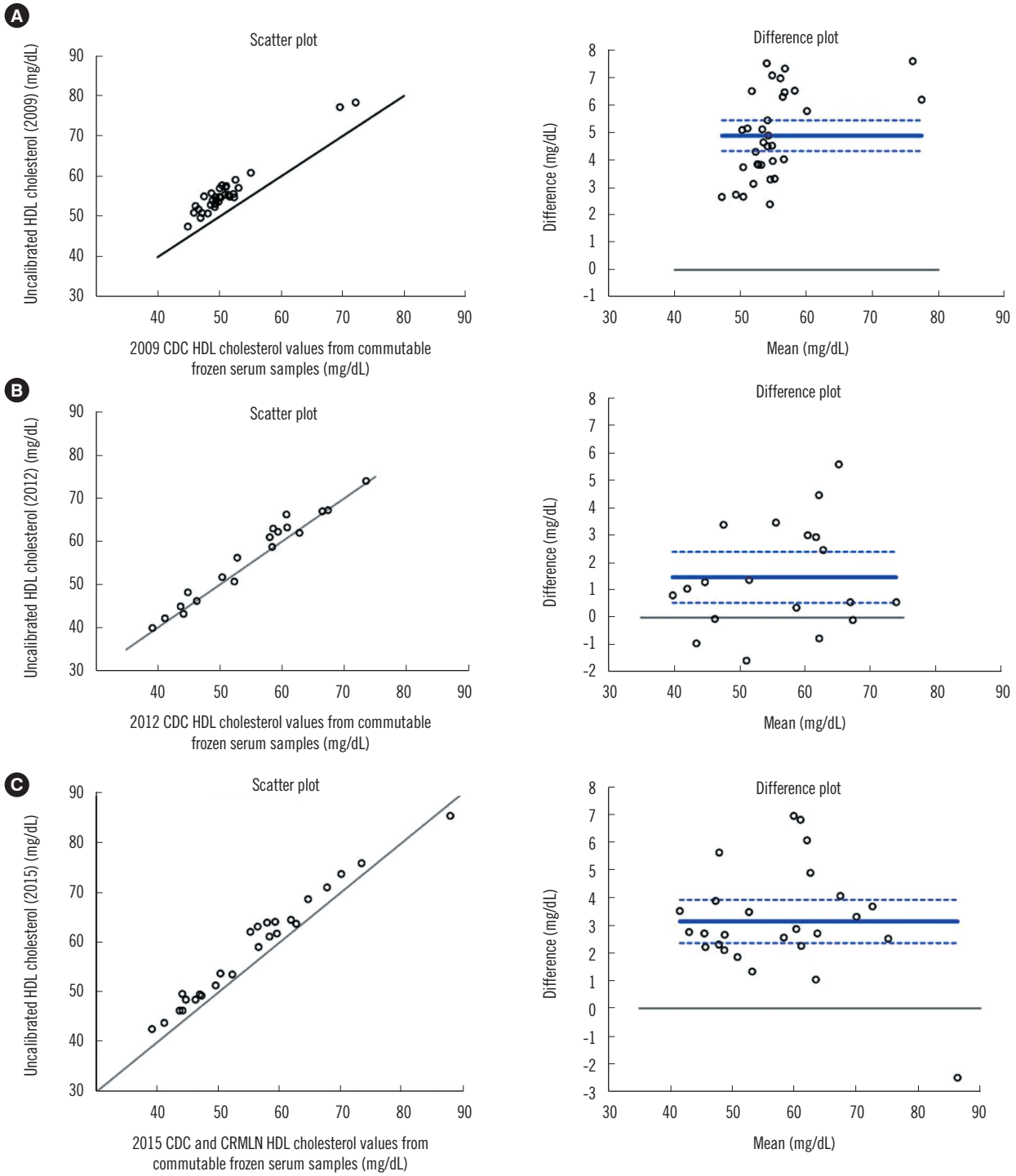

Fig. 2. High-density lipoprotein cholesterol (HDL-C) comparison study in 2009, 2012, and 2015. (A) Scatter plot and Bland-Altman plot of uncalibrated HDL-C (2009) and Centers for Disease Control and Prevention (CDC) reference laboratory HDL-C values from commutable frozen serum samples (mg/dL). (B) Scatter plot and Bland-Altman plot of uncalibrated HDL-C (2012) and CDC reference HDL-C. (C) Scatter plot and Bland-Altman plot of uncalibrated HDL-C (2015) and CDC and Cholesterol Reference Method Laboratory Network (CRMLN) reference HDL-C values. The solid line in the scatter plot is the line of identity (45 degree). The solid line in the Bland-Altman plot is the mean difference with 95% confidence interval (dotted lines); the solid line is drawn at zero.

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Table 2. Determination of the need for correction of HDL-C value and its applicable period*

Year	Number of samples†	Mean HDL-C difference (%)‡	Mean HDL-C difference (%) corrected by each calibration equation§			Selected equation for correction‖
			2009 Equation	2012 Equation	2015 Equation	
2008	6	9.40	**0.41** (0.65)	6.38 (3.47)	4.02 (2.75)	2009 Equation
2009	6	8.98	**0.46** (1.46)	6.17 (2.95)	3.08 (1.85)	2009 Equation
2010	30	8.05	**-1.05** (1.52)	4.97 (2.89)	3.03 (2.35)	2009 Equation
2011	54	5.57	-3.00 (2.16)	2.71 (1.73)	**0.26** (1.25)	2015 Equation
2012	54	3.20	-5.05 (3.22)	**0.46** (1.15)	-2.15 (1.49)	2012 Equation
2013	48	2.85	-5.19 (3.41)	**0.20** (1.46)	-2.73 (1.87)	2012 Equation
2014	48	3.42	-4.84 (3.02)	**0.67** (1.31)	-1.97 (1.62)	2012 Equation
2015	24	4.72	-3.66 (2.44)	1.93 (1.30)	**-0.72** (1.03)	2015 Equation

*The analysis was based on the results of external quality assurance programs including the Lipid Standardization Program (LSP) of the Center for Disease Control and Prevention (third quarter 2010-second quarter 2015), and the Accuracy-Based Lipid Survey (ABL) of the College of American Pathologists (2008-2012) in a laboratory, in which uncalibrated HDL cholesterol measurements were performed for the Korea National Health and Nutrition Examination Survey (KNHANES) during 2008-2015; †The number of samples was counted by the number of results of external quality assurance programs (LSP and ABL) to calculate the mean HDL-C difference (%); ‡Calculation formula: (uncalibrated value - reference value)/reference value×100; §Calculation formula is same as mean HDL-C difference (%). Each calibration equation of 2009, 2012, and 2015 was derived from the comparison studies of the HDL-C values between the NHANES laboratory and the CRMLN laboratory. Standard error of the mean HDL-C difference corrected was presented in the parenthesis. Standard Error = $\sqrt{\frac{1}{n}\Sigma(\hat{y}_i - y_i)^2}$; ‖The calibration equation with the least standard error was selected for the correction each year.
Abbreviation: HDL-C, high-density lipoprotein cholesterol.

Table 3. Final calibration equations

		Intercept	+	Slope	×	Uncalibrated KNHANES HDL-C (mg/dL)
2009 Calibration equation	Standard HDL-C* =	2.460	+	0.872	×	KNHANES 2008, 2009 and 2010 uncalibrated HDL-C
2012 Calibration equation	Standard HDL-C* =	1.096	+	0.952	×	KNHANES 2012, 2013 and 2014 uncalibrated HDL-C
2015 Calibration equation	Standard HDL-C* =	-3.172	+	1.01	×	KNHANES 2011 and 2015 uncalibrated HDL-C

*Calibration equations were based on the serum HDL cholesterol (mg/dL) through calibration studies. The values of intercept and slope for each equation were calculated by converting the uncalibrated HDL-C (Y) and the CDC reference value (X) (mg/dL) from the original regression equations of the Passing-Bablok fit.
Abbreviations: KNHANES, Korea National Health and Nutrition Examination Survey; HDL-C, high-density lipoprotein cholesterol.

obtained in each of these years. We reviewed the calibration equations from our 2009, 2012, and 2015 studies and selected the equation with the least mean difference for each year. The 2009 calibration equation was chosen to correct KNHANES data from 2008, 2009, and 2010. We chose the 2015 equation to correct KNHANES data from 2011 and 2015. Relatively smaller positive biases were observed in 2012, 2013, and 2014; the lowest bias was observed in 2013 (mean bias, 2.85%). For these three years, we used the 2012 equation. We created the following final calibration equations to correct HDL-C values from 2008 to 2015 on the basis of Passing-Bablok regression analysis (Table 3).

3. Distribution of the HDL-C values in KNHANES from 2008 to 2015

The scatter plots of original and calibrated HDL-C values of KNHANES for eight years (2008 to 2015) are presented in Fig. 3. We included all participants aged 30 yr or older in the final analysis. The original values were closer to the reference values once corrected by the calibration equations. Over the 8-yr period, the corrected HDL-C values tended to increase.

DISCUSSION

In this study, the uncalibrated KNHANES HDL-C values from 2008 to 2015 showed substantial positive biases compared with the CDC reference method values. Especially, those from 2008 to 2011 showed biases greater than 5% of the NCEP inaccuracy criterion for lipid testing (5.57-9.40%). The accuracy criterion for the CRMLN, which uses reference methods or designated comparison methods that are rigorously standardized to the CDC reference methods, is ≤1 mg/dL, regardless of the HDL-C level. This degree of biases in HDL-C measurements would yield large differences in hypoalphalipoproteinemia estimates based on KNHANES data. Especially, many individuals' HDL-C values

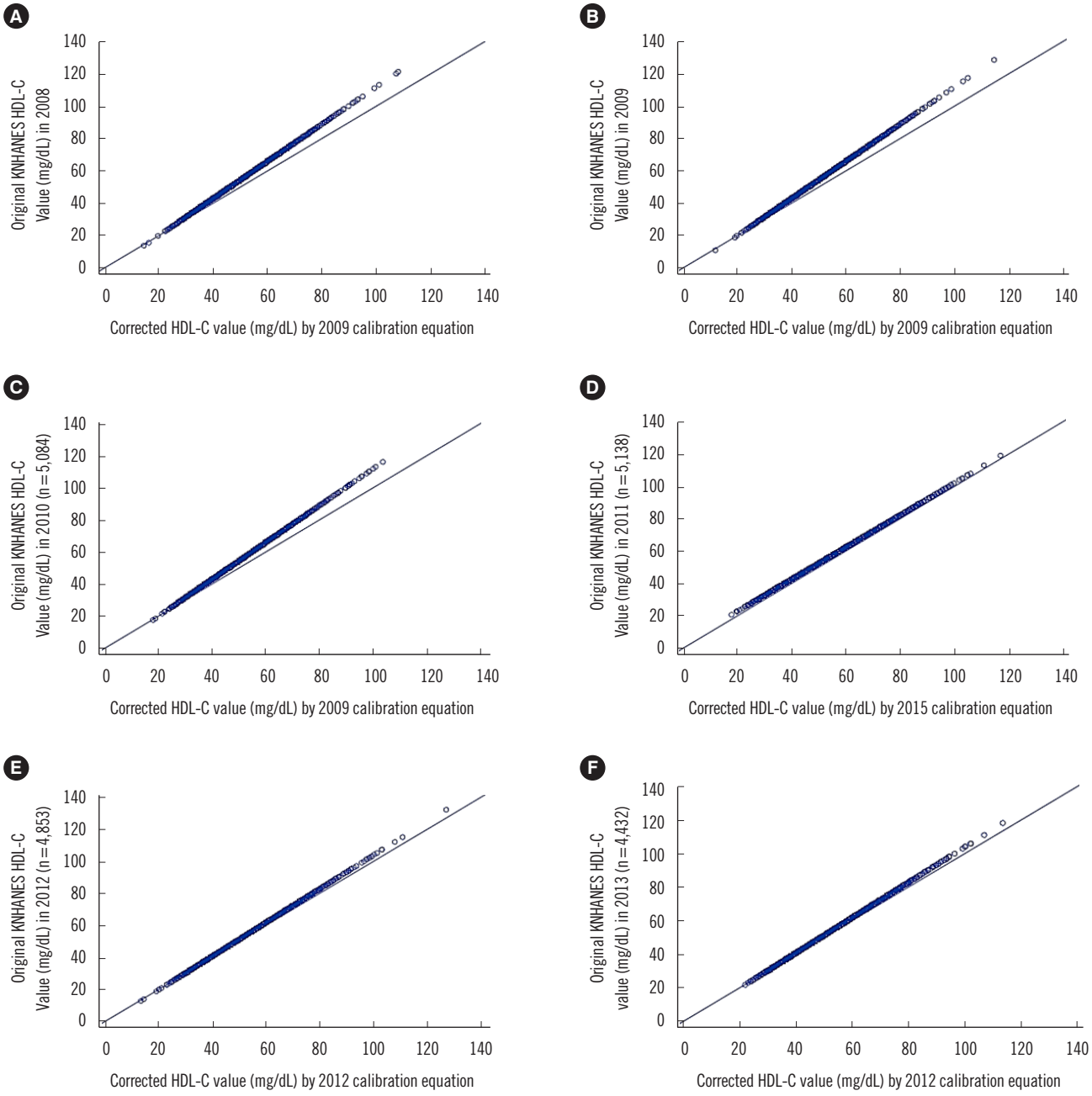

Fig. 3. Distribution of Korea National Health and Nutrition Examination Survey (KNHANES) 2008-2015 data for each year before and after data correction by calibration, expressed as a scatter plot. Calibration equations were used based on Passing-Bablok regression analysis. The mean value (mg/dL) of the original KNHANES HDL-C data with the corrected values by calibration equation in each year is shown (n is the number of results for the KNHANES participants above 30 yr of age). (A) The mean value of 50.6 (mg/dL) in KNHANES 2008 HDL-C data (n=5,530) was corrected to 47.6 (mg/dL) by the 2009 calibration equation. (B) The mean value of 51.3 (mg/dL) in KNHANES 2009 HDL-C data (n=6,001) was corrected to 47.2 (mg/dL) by the 2009 equation. (C) The mean value of 52.3 (mg/dL) in KNHANES 2010 HDL-C data (n=5,084) was corrected to 48.0 (mg/dL) by the 2009 equation. (D) The mean value of 52.5 (mg/dL) in KNHANES 2011 HDL-C data (n=5,138) was corrected to 49.8 (mg/dL) by the 2015 equation. (E) The mean value of 51.0 (mg/dL) in KNHANES 2012 HDL-C data (n=4,853) was corrected to 49.6 (mg/dL) by the 2012 equation. (F) The mean value of 51.5 (mg/dL) in KNHANES 2013 HDL-C data (n=4,432) was corrected to 50.2 (mg/dL) by the 2012 equation.
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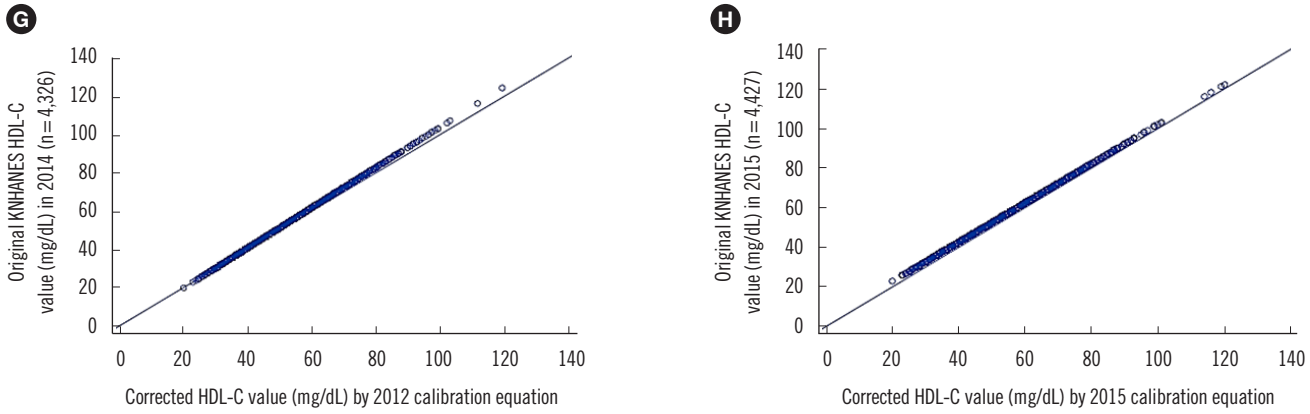

Fig. 3. (Continued) (G) The mean value of 52.0 (mg/dL) in KNHANES 2014 HDL-C data (n=4,326) was corrected to 50.6 (mg/dL) by the 2012 equation. (H) The mean value of 53.1 (mg/dL) in KNHANES 2015 HDL-C data (n=4,427) was corrected to 50.4 (mg/dL) by the 2015 equation.

were near the cut-off value of 40 mg/dL for hypoalphalipoproteinemia. Thus, correction of the original KNHANES HDL-C values is needed for appropriate estimation of hypoalphalipoproteinemia prevalence in the Korean population. A small bias in HDL-C values could make a large impact on the calculated prevalence; HDL-C value shift of 2 mg/dL could make a 5-7% shift in the calculated hypoalphalipoproteinemia prevalence in the Korean population according to the previous report of development of trend analysis for lipid profile in the KNHANES [8].

NHANES has been conducted in the United States by the CDC since 1971; corrected serum creatinine, cystatin C, HDL-C, and 25-hydroxyvitamin D values from NHANES data have been reported after correction with calibration equations [2, 3, 9, 10]. In case of the HDL-C analysis, the heparin-manganese precipitation method and direct immunoassay method for 1999-2000, 2001-2002, and 2005-2006 showed an undesirable bias (>4%) compared with the laboratory's HDL-C quality controls (Solomon Park Research Laboratories, Kirkland, WA, USA) that were assigned values established by the CDC. The HDL-C for 1999-2000, 2001-2002, and 2005-2006 were adjusted by the equation based on the comparison between the quality control HDL-C value associated with each participant sample and its corresponding Solomon Park-assigned HDL-C value [10].

In July 2015, we evaluated the bias of the direct HDL-C assay (Sekisui Medical Co.) of the KNHANES laboratory (Seegene Medical Foundation) compared with the reference method values determined by the CRMLN laboratory at the CEQAL Inc. If the 2015 KNHANES HDL-C values had no bias compared with the CDC reference method values, calibration equations could have been derived from remeasurement of the original KNHANES samples on the basis of a regression analysis between the original values and the remeasured values. However, since the comparison study for 2015 KNHANES HDL-C showed a positive bias of 4.72%, we attempted to determine a calibration equation based on previous comparison studies of HDL-C values of the KNHANES laboratory with the CDC reference method values.

We performed three comparison studies in 2009, 2012, and 2015. Since three calibration equations were only available in our study, we used the accuracy-based EQA results of the KNHANES laboratory to select the appropriate equation for correcting KNHANES HDL-C data in each year from 2008 to 2015. The accuracy-based EQA programs in our study were the LSP of the CDC and the accuracy-based lipid survey of the CAP, whose reference method values were oriented from the CDC reference method performed by the Lipid Reference Laboratory, Clinical Chemistry Branch, CDC or by the CRMLN laboratory at the Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington. Since we aimed to calibrate KNHANES HDL-C data to the CDC reference method values, the way to select calibration equation based on the evaluation data for the accuracy-based EQA programs referred to the CDC reference method values could work for our purpose. We calculated mean HDL-C difference (%) using the formula (uncalibrated value - reference value)/reference value×100). The appropriate calibration equation in each year was selected on the basis of the least standard error of mean HDL-C difference corrected by each calibration equation (Table 2).

Our study has some limitations. As at least 24 mL of CFS is needed to obtain a reference value using the CDC HDL-C reference method [4-6], we could not ensure a sufficient number of samples for the comparison studies (33 samples in 2009, 19 samples in 2012, and 26 samples in 2015, respectively). The

CLSI guideline EP09-A3 recommends the use of at least 40 samples to estimate bias to improve confidence in the statistical estimates and increase the opportunity to incorporate the effects of unexpected interfering substances (individual idiosyncratic biases) [11]. Therefore, the small number of samples for comparison may be a limitation to deriving adequate calibration equations for data correction. Including the limitation of small sample number in the comparison, our suggestion of using calibration equations based on the comparison studies for correction should be considered as one of the ways to calibrate KNHANES data. Further studies would be needed to find a better way.

In summary, our results provide a basis for HDL-C data correction from the KNHANES and provide a calibration equation to ensure standardization. The recommended equation for correcting the original HDL-C values obtained from KNHANES in 2008-2015 can be used to eliminate bias in the originally reported measurements that resulted from drift in the calibration of the measurement procedures. However, since the need to correct biases could be changed by the characteristics of research topics, the decision to convert the KNHANES data using these equations should be decided on an individual basis in each study.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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