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Assessing the Practical Differences in LDL-C Estimates Calculated by Friedewald, Martin/Hopkins, or NIH Equation 2: An Observation Cross-Sectional Study

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ABSTRACT

Objective: Low-density lipoprotein-cholesterol (LDL-C) remains a clinically important cholesterol target in primary prevention of atherosclerotic cardiovascular disease. The present study aimed to assess the practical differences among three equations utilized for the estimation of LDL-C: the Friedewald, the Martin/Hopkins, and the NIH equation 2.

Methods: Blood lipid measurements from 4,556 noninstitutionalized participants, aged 12 to 80, were obtained from the 2017-2020 National Health and Nutrition Examination Survey study. We 1) assessed the differences between three calculated LDL-C estimates, 2) examined the correlations between LDL-C estimates using correlation coefficients and regression, and 3) investigated the degree of agreement in classifying individuals into the LDL-C category using weighted Kappa and percentage of agreement.

Results: The differences in LDL-C estimates between equations varied by sex and triglyceride levels ($p<0.001$). Overall, the mean of absolute differences between Friedewald and Martin/Hopkins was 3.17 mg/dL (median=2.0, 95% confidence interval [CI] [3.07–3.27]). The mean of absolute differences between Friedewald and NIH Equation 2 was 2.08 mg/dL (median=2.0, 95% CI [2.03–2.14]). Friedewald correlated highly with Martin/Hopkins ($r=0.991$, rho=0.989) and NIH Equation 2 ($r=0.998$, rho=0.997). Cohen's weighted Kappa=0.92 between Friedewald and Martin/Hopkins, and 0.95 between Friedewald and NIH equation 2. The percentage of agreement in classifying individuals into the same LDL-C category was 93.0% between Friedewald and Martin/Hopkins, and 95.4% between Friedewald and NIH equation 2.

Conclusion: Understanding the practical differences in LDL-C calculations can be helpful in facilitating decision-making during a paradigm shift.

Keywords: Lipids; Aging; Atherosclerosis; Cholesterol; Cardiovascular disease; Hypercholesterolemia

INTRODUCTION

Low-density lipoprotein-cholesterol (LDL-C) remains a key contributor to plaque formation and remains of utmost clinical importance as a cholesterol target in the primary prevention of atherosclerotic cardiovascular disease. Dyslipidemia guidelines require follow-up testing of LDL-C to determine treatment adequacy and assess percent LDL-C lowering.[1-3] While direct LDL-C assays via beta-quantification in the clinical laboratory are considered the gold standard for LDL-C measurement, they are often technique-sensitive, labor-intensive, time-consuming, and expensive, making them unsuitable for general use.[4] As such, Friedewald's equation,[5] a mathematical equation developed in 1972, has been utilized for estimating LDL-C concentration in high-volume routine laboratories due to its ease of use, convenience, cost-effectiveness, and reasonably good accuracy.

The Friedewald equation estimated LDL-C as: total cholesterol (TC) minus high-density lipoprotein-cholesterol (HDL-C) minus triglycerides (TG)/5, with the latter term serving as an estimate for very low-density lipoprotein-cholesterol (VLDL-C). Notwithstanding, the Friedewald calculation suffers from some application limitations. This calculation was recommended when TGs were <400 mg/dL. The LDL-C estimates using the Friedewald equation are prone to inaccuracies with increasing TG and decreasing LDL-C concentrations, especially when the TG levels ≥400 mg/dL, or LDL-C levels <70 mg/dL.[6-8] Under these scenarios, patients who have their LDL-C underestimated may lead to delay the initiation of adequate lipid-lowering therapy in high-risk patients such as hypertriglyceridemia and hypo-HDL-cholesterolemia.[9-11] On the other hand, patients who have their LDL-C overestimated at higher levels may result in unnecessary pharmacological therapy.[7]

With the increased prevalence of high TG states such as in people with diabetes, the cut-off LDL-C levels or % LDL-C reduction described in the AHA/ACC[2] and ESC/EAS Guidelines,[3] and lower LDL-C levels are targeted by novel lipid-lowering agents such as PCSK9 inhibitors, bempedoic acid, and ezetimibe, the expert panel acknowledged the importance of accurate LDL-C estimation. More accurate, timely, and relatively economic methods in measuring LDL-C levels are warranted, either using a direct LDL-C assay and/or an alternative LDL-C equation. Over recent years, many new estimation methods have been proposed.[12-23] Among them, two equations have growing support: Martin/Hopkins equation[24] and the NIH equation 2.[25]

Many external validation studies have been conducted and showed superiority of the Martin/Hopkins[11,24,26-36] or NIH Equation 2[25,27,37,38] in relation to the Friedewald formula. Here, the purpose of this study was to assess the practical differences[39,40] in LDL-C estimates calculated by Friedewald, Martin/Hopkins, or NIH equation 2 in a population-based, random-sampled, noninstitutionalized general U.S. sample. We 1) compared the score distribution differences between Friedewald and Martin/Hopkins calculated LDL-C estimates, and between Friedewald and NIH equation 2 calculated LDL-C estimates, 2) examined the correlations between LDL-C estimates using the correlation coefficients and regression analysis, and 3) investigated the degree of agreement in classifying individuals into the LDL-C category using weighted Kappa and the percentage of agreement. Understanding the practical differences in LDL-C calculations can be helpful in facilitating decision-making during a paradigm shift.

MATERIALS AND METHODS

1. Study design

Written informed consent was obtained from participants aged 12 years and older and written child assent was obtained from those aged 7 to11 years. Approval for use of the NHANES data for this study was provided by the NCHS Research Ethics Review Board. Because this study involved secondary analysis of de-identified data, the Institutional Review Boards of the University of Wisconsin - Milwaukee determined that this study did not fall within the regulatory definition of research involving human subjects and did not require further institutional review board review.

This was an observational cross-sectional study. We used 2017-2020 data from the National Health and Nutrition Examination Survey (NHANES), which is a major program of the National Center for Health Statistics (NCHS), part of the Centers for Disease Control and Prevention (CDC). The NHANES data collection methodology is based on complex, multistage probability samples of the U.S. noninstitutionalized population, and is intended to be nationally representative. The NHANES data were collected via in-person interviews, which took place in the participants' homes, followed by a physical assessment conducted at a mobile examination center. Before the blood draw, the phlebotomist assessed the participant's fasting status. As part of the laboratory assessment, the serum TC and HDL-C levels were measured for participants aged 6 years and older, while fasting TGs were measured for those aged 12 years and older.

2. Laboratory measurements

Blood samples were received frozen and stored at −80ºC in the freezer until testing was performed. Upon completion of the analysis, specimens were stored at −70ºC and discarded after 1 year. All lipid analyses were analyzed according to a standardized protocol and can be found on the NHANES website (https://wwwn.cdc.gov/nchs/nhanes). Through the past decade, there were no major changes to the laboratory methods. The blood specimen was analyzed using the Roche/Hitachi Cobas 6000 chemistry analyzer in recent years. However, the TC, HDL-C, and TGs were measured on the Roche modular P chemistry analyzer in the 2011–2012 cycle, and were measured on the Roche modular P and Roche Cobas 6000 chemistry analyzers in the 2013–2014 cycle.

3. LDL-C data

The LDL-C in mg/dL was calculated by Friedewald,[5] Martin/Hopkins,[24] or NIH equation 2.[25] The Friedewald equation was derived from a sample of 448 individuals with familial hyperlipoproteinemia or their relatives in 1972, the Friedewald equation uses three laboratory measures and estimates LDL-C as: TC − HDL-C − (TG)/5. A fixed factor of 5 was used to describe the relationship between TG and VLDL-C.

Derived from a nationally representative sample of 1.35 million patients with lipid distributions around 2013, the Martin/Hopkins estimated LDL-C as TC − HDL-C − (TG)/(novel factor). The novel factor is an adjustable factor based on an individual's non-HDL-C and TG levels and could be obtained from a 6 by 30 table (i.e., 180-cell). It is important to note that the primary intention behind developing the Martin/Hopkins equation was to improve LDL-C estimation accuracy at clinically relevant low LDL-C and moderately elevated TG (150–400 mg/dL) levels, not to replace the need for direct LDL-C measurement at TG ≥400 mg/dL. A good deal of external validations confirm that Martin/Hopkins equation outperforms others in accuracy.[11,24,26-36]

In 2020, the NIH equation 2 was derived from 18,715 lipid samples from 8,656 patients at the NIH collected between 1970s and 1990s. The NIH equation 2 estimates LDL-C as: LDL-C = TC/0.948 – HDL-C/0.971 – (TG/8.56 + [TG x NonHDL-C]/2140 – TG^2/16100) – 9.44. Several studies[25,27,37,38] have demonstrated that the new equation is more accurate than other LDL-C equations in patients with hypertriglyceridemia, with a reduced rate of misclassifications. The NIH equation gives valid LDL-C results with TG concentrations up to 800 mg/dL.[25]

For LDL-C calculated according to the Friedewald equation, Martin/Hopkins equation, or NIH equation 2, data were labeled 'LDL-F' for Friedewald, 'LDL-M' for Martin/Hopkins, and 'LDL-N' for NIH equation 2.

4. Statistical analyses

To evaluate differences in the overall score distribution between estimated LDL-C values, we first used General Linear Model (GLM) with repeated measures (i.e., LDL-F vs. LDL-M, LDL-F vs. LDL-N). As cholesterol data is often skewed, differences between LDL-C estimates were further assessed using nonparametric Wilcoxon Signed Rank Test and Bland-Altman plots. Additionally, we conducted subgroup analyses based on LDL-C levels and TG levels, and presented the percentage of sample that had differences less than 5 mg/dL and 10 mg/dL. For the correlations between LDL-F, LDL-M, and LDL-N, we used Pearson (r) and Spearman's (rho) correlation coefficients, followed by linear regression (R^2). To assess the practical difference in interpreting cholesterol results, weighted Kappa was used to assess the degree of agreement in classifying individuals into the LDL-C category: optimal (less than 100 mg/dL), near or above optimal (100–129 mg/dL), borderline high (130–159 mg/dL), high (160–189 mg/dL), and very high (190 through highest). We calculated the percentage of agreement in classifying individuals into the same LDL-C category, with a difference of one level, as well as with a difference of two levels. Results from both parametric and nonparametric tests were presented to provide a more complete picture of the data. The significant level was set at alpha=0.05. All analyses were performed using the SPSS Statistics for Windows, Version 28.0. (IBM Corp., Armonk, NY, USA).

RESULTS

1. Analytic sample

Data for analysis included 4,556 participants aged 12 to 80+ years old. The mean (SD) age was 45.8 (20.6). Among them, 51.4% of the sample were females. The majority of the sample was non-Hispanic white (33.4%), followed by non-Hispanic black (25.1%), others including multi-racial (17.7%), Mexican American (13.5%), and other Hispanic (10.2%). About 29.5% of the sample were overweight, followed by normal (27.6%), obsess class 1 (19.4%), obese class 2 (9.8%), obese class 3 (8.6%), and underweight (3.2%) (1.8% did not have body mass index data). More detailed baseline characteristics of participants is available in **Supplementary Table 1**.

2. Score distribution differences

The mean (SD) LDL-C for Friedewald-, Martin/Hopkins-, and NIH equation 2-calculated LDL-C estimates (i.e., LDL-F, LDL-M, and LDL-N) was 105.4 (35.2), 105.7 (35.2), and 106.9 (35.9), respectively. The median and interquartile range (IQR) was 102 (IQR=45), 102 (IQR=46), and 103 (IQR=46) for LDL-F, LDL-M, and LDL-N, respectively. **Table 1** summarizes descriptive statistics of LDL-C estimates among the Friedewald, Martin/Hopkins, and NIH Equation 2 equations.

Table 1. Descriptive statistics of LDL-C estimates among the Friedewald, Martin/Hopkins, and NIH equation 2 equations by triglyceride

Variables	Values					Percentiles			Wilcoxon
	No.	Mean	SD	Min	Max	Q1	Q2	Q3	*p*-value
Entire data									
LDL-F	4,556	105.4	35.2	7	357	81.0	102.0	126.0	
LDL-M	4,556	105.7	35.2	14	358	81.0	102.0	127.0	<0.001
LDL-N	4,556	106.9	35.9	14	359	82.0	103.0	128.0	<0.001
TG (mg/dL), <25									
LDL-F	48	69.4	25.1	25	137	50.8	63.5	88.8	
LDL-M	48	67.3	25.0	24	134	48.5	62.0	86.8	<0.001
LDL-N	48	66.7	26.4	21	138	47.5	59.5	87.0	<0.001
TG (mg/dL), 25–49									
LDL-F	753	89.4	26.9	22	224	70.5	86.0	106.0	
LDL-M	753	85.6	26.7	18	218	67.0	83.0	102.0	<0.001
LDL-N	753	88.4	28.0	18	228	69.0	85.0	106.0	<0.001
TG (mg/dL), 50–99									
LDL-F	1,983	104.2	32.7	15	270	81.0	100.0	123.0	
LDL-M	1,983	102.5	32.1	14	267	80.0	99.0	121.0	<0.001
LDL-N	1,983	104.9	33.4	14	275	81.0	101.0	124.0	<0.001
TG (mg/dL), 100–149									
LDL-F	1,038	114.4	36.4	15	357	89.0	113.0	136.0	
LDL-M	1,038	115.8	35.1	19	354	92.0	114.0	136.0	<0.001
LDL-N	1,038	117.0	36.3	18	359	92.0	115.5	138.0	<0.001
TG (mg/dL), 150–199									
LDL-F	419	115.1	38.1	30	247	88.0	114.0	140.0	
LDL-M	419	120.4	35.9	38	247	95.0	120.0	144.0	<0.001
LDL-N	419	119.1	37.1	35	247	92.0	118.0	143.0	<0.001
TG (mg/dL), 200–249									
LDL-F	168	118.5	44.4	24	354	86.3	116.0	143.8	
LDL-M	168	127.8	41.5	38	358	98.0	124.0	151.0	<0.001
LDL-N	168	123.8	42.3	33	349	92.3	120.5	148.0	<0.001
TG (mg/dL), 250–299									
LDL-F	82	113.7	38.9	55	219	85.0	109.5	138.0	
LDL-M	82	127.3	35.3	71	224	101.8	123.0	148.5	<0.001
LDL-N	82	120.3	36.2	64	219	94.0	116.5	142.8	<0.001
TG (mg/dL), 300–349									
LDL-F	36	106.1	46.9	7	198	68.3	99.0	138.0	
LDL-M	36	124.4	40.8	42	209	91.3	117.5	151.0	<0.001
LDL-N	36	114.6	42.5	27	198	80.0	108.0	143.3	<0.001
TG (mg/dL), 350–399									
LDL-F	29	106.1	36.6	35	186	71.5	104.0	129.5	
LDL-M	29	127.6	31.8	65	197	99.0	128.0	148.0	<0.001
LDL-N	29	115.7	32.3	52	186	86.0	114.0	136.0	<0.001

Q1 is the 25th percentile, meaning that 25% of the data falls below the first quartile. Q2 (or the median) is the 50th percentile. Q3 is the 75th percentile.
LDL-C, low-density lipoprotein-cholesterol; SD, standard deviation; Min, minimum; Max, maximum; LDL-F, Friedewald-calculated LDL-C (mg/dL); LDL-M, Martin/Hopkins-calculated LDL-C (mg/dL); LDL-N, NIH equation 2-calculated LDL-C (mg/dL); TG, triglyceride.

GLM results supported that there were statistical differences in LDL-C estimates between LDL-F and LDL-M (*p*<0.001), and between LDL-F and LDL-N (*p*<0.001). Results from the Wilcoxon Signed-Ranks test were consistent, indicating the discrepancy between LDL-F and LDL-M (*p*<0.001) and between LDL-F and LDL-N (*p*<0.001).

The differences in LDL-C estimates between equations varied by sex and TG levels (*p*<0.001). Overall, the mean of absolute differences between LDL-F and LDL-M was 3.17 mg/dL (95% confidence interval [CI] [3.07, 3.27], Q1=1.0, median=2.0, Q3=4.0), with the smallest mean difference of 1.36 mg/dL (when TG=100–149 mg/dL) and largest difference of 21.57 mg/dL (when TG=350–399 mg/dL). About 89.0% had differences between LDL-F and LDL-M within 5 mg/dL, and 95.7% were within 10 mg/dL.

The mean of absolute differences between LDL-F and LDL-N was 2.08 mg/dL (95% CI [2.03, 2.14], Q1=1.0, median=2.0, Q3=3.0), with the smallest mean difference of 0.64 mg/dL (when TG=50–99 mg/dL) and largest difference of 9.75 mg/dL (when TG=350–399 mg/dL). About 95.0% had differences between LDL-F and LDL-N within 5 mg/dL, and 99.4% were within 10 mg/dL. **Fig. 1** presents the Bland–Altman plot of LDL-C levels between Friedewald-calculated and Martin/Hopkins-calculated LDL-C estimates, and between Friedewald-calculated and NIH equation 2-calculated LDL-C estimates, stratified by sex.

Table 2 presents differences in LDL-C estimates between the Friedewald, Martin/Hopkins, and NIH Equation 2 by sex and TG levels. **Fig. 2** shows modified Bland–Altman plot of LDL-C levels between the Friedewald, Martin/Hopkins, and NIH equation 2 equations by sex and
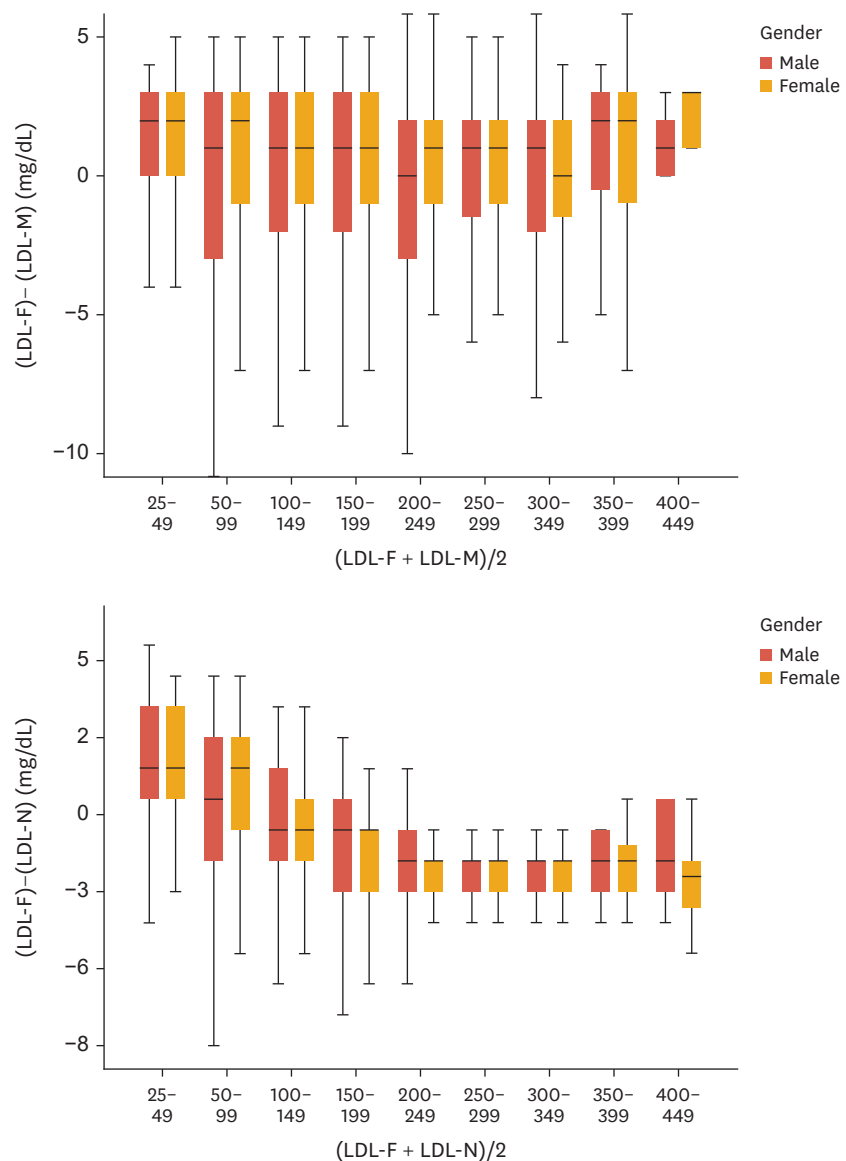

Fig. 1. Bland–Altman plot of LDL-C levels between Friedewald-calculated and Martin/Hopkins-calculated LDL-C estimates, and between Friedewald-calculated and NIH equation 2-calculated LDL-C estimates, stratified by sex. LDL-F, Friedewald-calculated LDL-C estimates; LDL-M, Martin/Hopkins-calculated LDL-C estimates; LDL-N, NIH equation 2-calculated LDL-C estimates; LDL-C, low-density lipoprotein-cholesterol.

Table 2. Differences in LDL-C estimates between the Friedewald, Martin/Hopkins, and NIH equation 2 by sex and triglyceride levels

Triglyceride level (mg/dL)	No.	(LDL-F) - (LDL-M) (mg/dL)				(LDL-F) - (LDL-N) (mg/dL)				(ELDL-M) - (LDL-N) (mg/dL)			
		Mean	SD	Median	95% CI	Mean	SD	Median	95% CI	Mean	SD	Median	95% CI
Sex													
Male													
<25	22	2.23	0.69	2	1.5, 3.0	2.82	1.50	3.5	2.3, 3.4	0.59	1.50	1	−0.1, 1.2
25–49	350	3.80	0.69	4	3.6, 4.0	1.09	1.28	1	1.0, 1.2	−2.71	1.68	−3	−2.9, −2.5
50–99	923	1.59	1.26	2	1.5, 1.7	−0.64	1.08	−1	−0.7, −0.6	−2.23	1.51	−2	−2.3, −2.1
100–149	507	−1.39	1.73	−1	−1.5, −1.2	−2.43	0.73	−2	−2.5, −2.3	−1.04	1.52	−1	−1.2, −0.9
150–199	219	−5.34	2.75	−5	−5.6, −5.1	−3.93	1.22	−4	−4.1, −3.8	1.41	1.74	1	1.2, 1.6
200–249	98	−9.61	3.41	−10	−10.0, −9.3	−5.46	1.97	−5	−5.7, −5.2	4.15	1.71	4	3.8, 4.5
250–299	50	−14.30	4.00	−15	−14.8, −13.8	−7.16	2.57	−7	−7.5, −6.8	7.14	1.77	7	6.7, 7.6
300–349	25	−19.68	6.88	−20	−20.4, −19.0	−9.24	4.71	−9	−9.7, −8.7	10.44	2.47	10	9.8, 11.1
350–399	21	−21.57	5.61	−23	−22.3, −20.8	−9.57	4.63	−9	−10.1, −9.0	12.00	1.73	12	11.3, 12.7
Female													
<25	26	2.00	0.49	2	1.3, 2.7	2.58	1.42	3	2.1, 3.1	0.58	1.75	1	0.0, 1.2
25–49	403	3.80	0.71	4	3.6, 4.0	0.88	1.18	1	0.8, 1.0	−2.92	1.55	−3	−3.1, −2.8
50–99	1,060	1.74	1.19	2	1.6, 1.9	−0.84	1.12	−1	−0.9, −0.8	−2.59	1.53	−2	−2.7, −2.5
100–149	531	−1.36	1.81	−1	−1.5, −1.2	−2.63	0.74	−3	−2.7, −2.5	−1.27	1.52	−1	−1.4, −1.1
150–199	200	−5.24	2.57	−5	−5.5, −5.0	−4.07	1.16	−4	−4.2, −3.9	1.17	1.64	1	1.0, 1.4
200–249	70	−8.79	3.42	−9	−9.2, −8.4	−4.91	2.44	−5	−5.2, −4.6	3.87	1.71	4	3.5, 4.2
250–299	32	−12.38	3.75	−13	−13.0, −11.7	−5.75	2.90	−7	−6.2, −5.3	6.63	1.41	7	6.1, 7.2
300–349	11	−15.27	4.10	−16	−16.3, −14.2	−6.73	3.58	−8	−7.5, −6.0	8.55	1.69	9	7.6, 9.5
350–399	8	−21.25	3.77	−21.5	−22.5, −20.0	−9.75	3.92	−10.5	−10.6, −8.9	11.50	1.20	12	10.4, 12.6

Measurement unit is in mg/dL.
LDL-C, low-density lipoprotein-cholesterol; LDL-F, Friedewald-calculated LDL-C (mg/dL); LDL-M, Martin/Hopkins-calculated LDL-C (mg/dL); LDL-N, NIH equation 2-calculated LDL-C (mg/dL); SD, standard deviation; CI, confidence interval.

TG levels. The differences of LDL-C estimates were prone to be larger with increasing TG concentrations. Furthermore, a moderate correlation was observed between absolute values of (LDL-F minus LDL-M), and absolute values of (LDL-F minus LDL-N) (r=0.771, *p*<0.001). As the differences between LDL-F and LDL-M increased, there was a corresponding increase in the differences between LDL-F and LDL-N (**Fig. 3**).

3. Correlations
Friedewald's LDL-F correlated highly with Martin/Hopkins's LDL-M (Pearson r=0.991, Spearman rho=0.989, R^2=0.982) and with NIH Equation 2's LDL-N (r=0.998, rho=0.997, R^2=0.996). **Fig. 4** presents scatter plots relating LDL-F, LDL-M, and LDL-N cholesterol with the regression coefficients.

4. Degree of agreement
Table 3 presents the degree of agreement between three equations. The degree of agreement was high: Cohen's weighted Kappa=0.92 between Friedewald and Martin/Hopkins, and 0.95 between Friedewald and NIH equation 2. Percentage of agreement in classifying individuals into the same LDL-C category, with a difference of one level, as well as with a difference of two levels was 93.0%, 7.1% and 0.0% between Friedewald and Martin/Hopkins, and was 95.4%, 4.6% and 0.0% between Friedewald and NIH equation 2.

DISCUSSION

The present study assessed the practical differences in LDL-C estimates calculated by Friedewald, Martin/Hopkins, or NIH equation 2. It is important to emphasize that this study was based on multistage probability samples of the U.S. noninstitutionalized population,
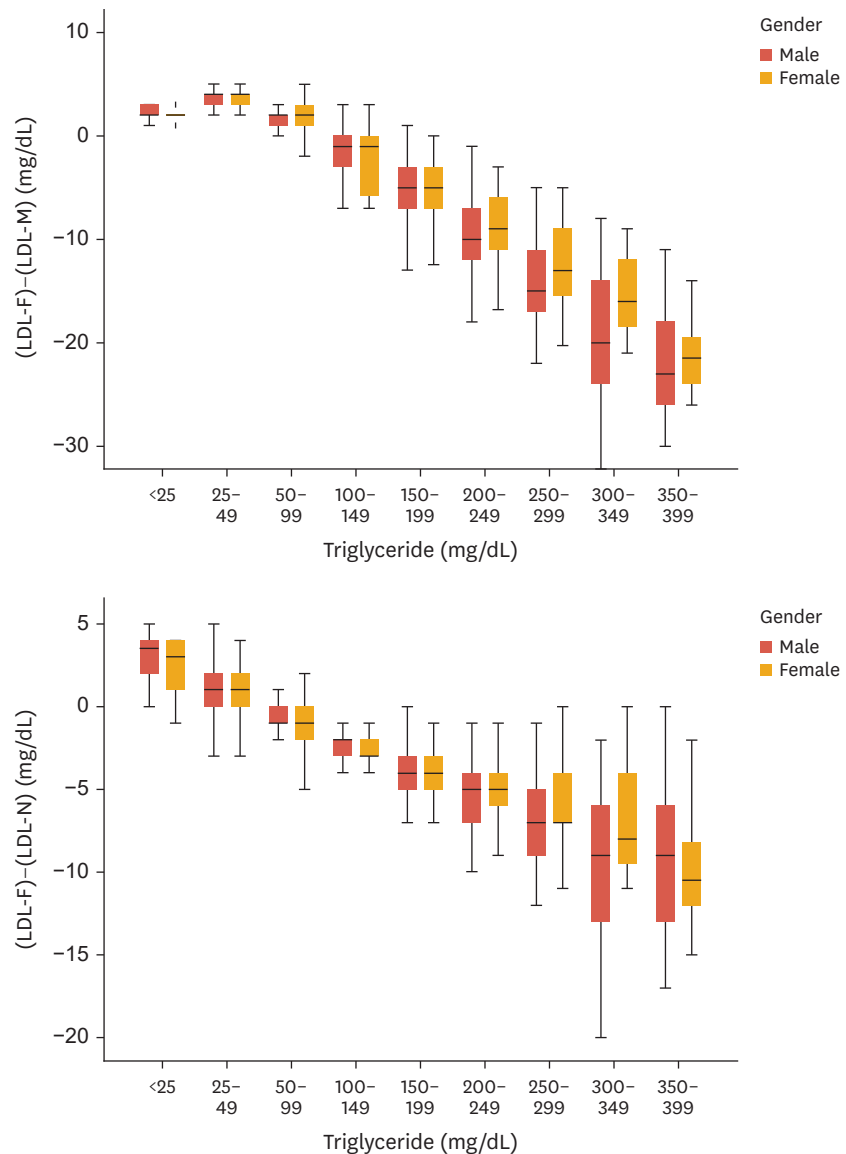
Fig. 2. Modified Bland–Altman plot of LDL-C levels between Friedewald-calculated and Martin/Hopkins-calculated LDL-C estimates, and between Friedewald-calculated and NIH equation 2-calculated LDL-C estimates, stratified by sex and triglyceride levels.
LDL-F, Friedewald-calculated LDL-C estimates; LDL-M, Martin/Hopkins-calculated LDL-C estimates; LDL-N, NIH equation 2-calculated LDL-C estimates; LDL-C, low-density lipoprotein-cholesterol.

aged 12 to 80+ who fasted before the blood test. While statistically significant, the discrepancy between Friedewald-calculated, Martin/Hopkins-calculated, or NIH equation 2-calculated LDL-C values was small and clinical insignificant at the group level. The results are in agreement with those of previous studies[26,38,41] indicating that Friedewald-calculated LDL-C provides a reasonable estimation of LDL-C and can guide treatment decisions for most patients. One may argue that the practical differences are critical at the individual level. However, evidence suggested that all three methods are not perfect when TG is high.[27,38,42,43]

The LDL-C differences between Friedewald and NIH equation 2 were relatively smaller than those between Friedewald and Martin/Hopkins. Several studies[25,27,37,38] have demonstrated that the NIH equation 2 is more accurate than other LDL-C equations. Still, Sampson et al.[25]
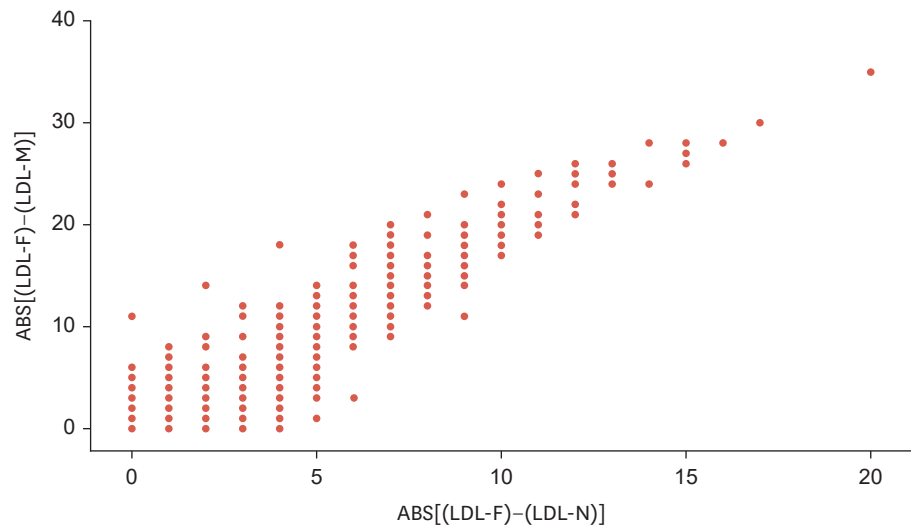
Fig. 3. Scatter plots between the ABS values of LDL-F minus LDL-M, and LDL-F minus LDL-N.
ABS, absolute; LDL-F, Friedewald-calculated LDL-C estimates; LDL-M, Martin/Hopkins-calculated LDL-C estimates; LDL-N, NIH equation 2-calculated LDL-C estimates; LDL-C, low-density lipoprotein-cholesterol.

reported a mean absolute deviation of 24.9 mg/dL for patients with hypertriglyceridemia when compared to the beta-quantification. In Ginsberg et al.'s study,[38] NIH equation 2 provided greater accuracy than Friedewald or Martin-Hopkins when TGs were >250 mg/dL, although inaccuracies were observed with all three methods. Higgins et al.[37] recommended clinical implementing the NIH equation for all patients except those with type III hyperlipoproteinemia.

Numerous external validations have demonstrated that the Martin/Hopkins equation surpasses other methods in terms of accuracy.[11,24,26–36] However, according to data presented by Martin et al.,[24] the overall concordance in guideline risk classification with directly measured LDL-C was 91.7% for patients with TGs lower than 400 mg/dL. Another study conducted by Martin et al.[11] found that the median difference between Martin/Hopkins LDL-C values and those obtained through preparative ultracentrifugation was −2 mg/dL

Table 3. The degree of agreement between three equations

Variables	LDL-F				
	Optimal	Near or above optimal	Borderline high	High	Very high
LDL-M					
Optimal	2,120	79	0	0	0
Near or above optimal	97	1,173	40	0	0
Borderline high	0	66	646	15	0
High	0	0	17	211	5
Very high	0	0	0	2	85
LDL-N					
Optimal	2,140	5	0	0	0
Near or above optimal	77	1,234	0	0	0
Borderline high LDL	0	79	668	0	0
High	0	0	35	216	0
Very high	0	0	0	12	90

LDL categories are defined as the following: Optimal LDL: less than 100 mg/dL; near or above optimal LDL: 100–129 mg/dL; Borderline high LDL: 130–159 mg/dL; high LDL: 160–189 mg/dL; very high: LDL 190 thru highest. LDL-C measurement unit is mg/dL.
LDL-C, low-density lipoprotein-cholesterol; LDL-F, Friedewald-calculated LDL-C estimates; LDL-M, Martin/Hopkins-calculated LDL-C estimates; LDL-N, NIH equation 2-calculated LDL-C estimates.
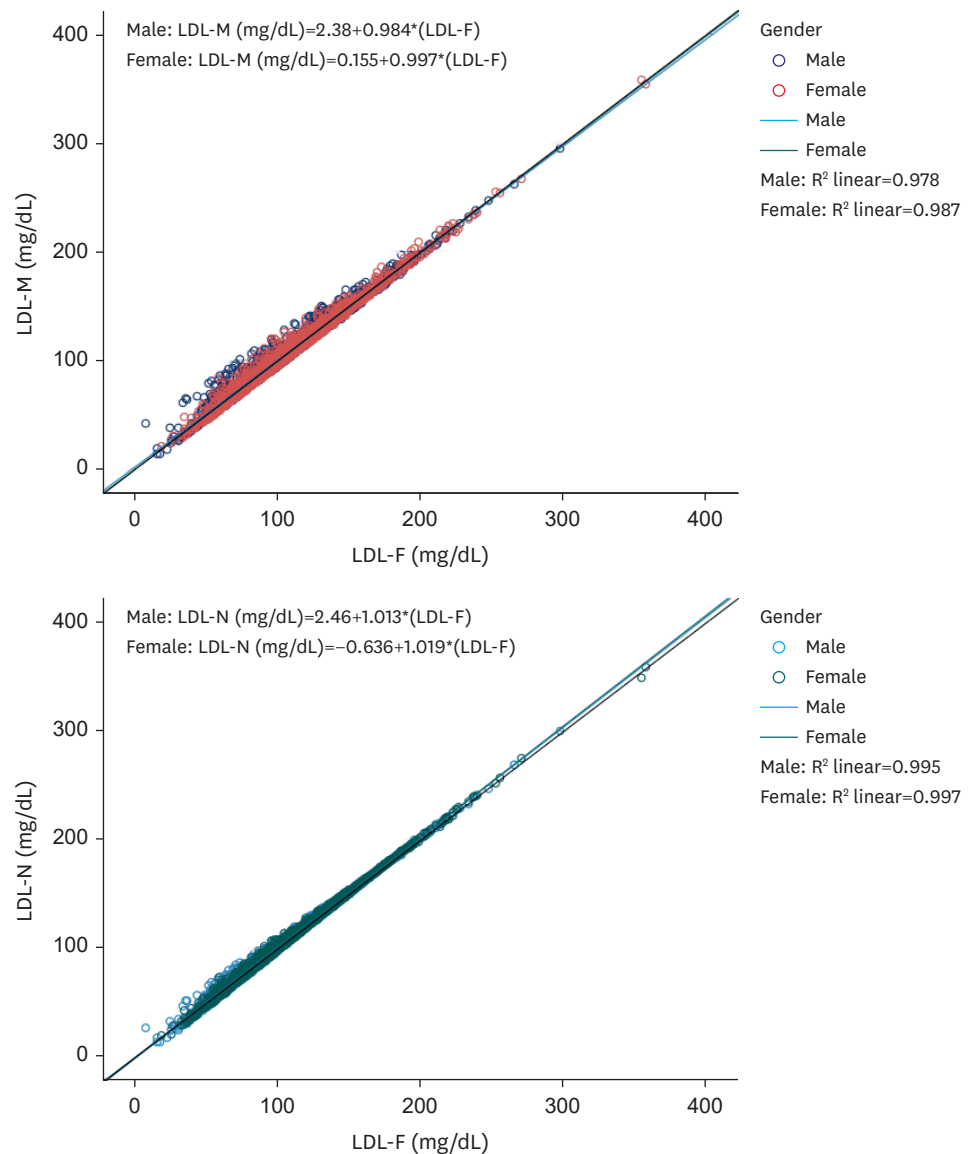
Fig. 4. Scatter plots relating LDL-F, LDL-M, and LDL-N cholesterol with the regression coefficients. LDL-C, low-density lipoprotein-cholesterol; LDL-M, Martin/Hopkins-calculated LDL-C estimates; LDL-F, Friedewald-calculated LDL-C estimates.

(IQR, −4 to 1 mg/dL) with 22.9% and 2.6% of Martin/Hopkins LDL-C values differing from preparative ultracentrifugation levels by more than 5 mg/dL and 10 mg/dL, respectively.

In this study, we compared the Friedewald equation with two alternative equations for estimating LDL under varying TG levels. Our findings suggest that differences between the equations were small when TG levels were low. We further investigated these differences by stratifying samples by TG levels and calculating the percentage of absolute differences between the Friedewald equation and the alternative equations. Our results revealed that, when comparing the Friedewald equation with the Martin/Hopkins equation, 100%, 99.3%, 100%, 98.7%, and 53.7% of the stratified subsamples were within 5 mg/dL differences when TG levels were at or below 25, 25–49, 50–99, 100–149, and 150–199 mg/dL, respectively. Similarly, when comparing the Friedewald equation with the NIH equation 2, we found

that 100%, 100%, 100%, 99.9%, and 89.5% of the stratified subsamples were within 5 mg/dL differences when TG levels were in the < 25, 25–49, 50–99, 100–149, and 150–199 mg/dL range, respectively. Our results suggest that a TG level of less than 149 mg/dL is an acceptable range, as there were no significant disagreements among the three equations. Within our dataset of 4,556 individuals, it was observed that approximately 83.9% of the data exhibited TG levels below 149 mg/dL. However, when TG levels exceeded 149 mg/dL, a greater number of samples showed differences of 5 mg/dL or greater among the three equations at the individual level. In this scenario, it may be appropriate to consider the use of alternative equations, such as the Martin/Hopkins or the NIH equation 2.

Here, we found high correlations and linear relationships between Friedewald and Martin/Hopkins, as well as Friedewald and NIH equation 2. This finding was consistent with results reported by Egbaria et al.[10] where they found a correlation coefficient (r) of 0.898 between Martin/Hopkins and Friedewald LDL-C estimations. Besides, several studies have shown strong correlations and linear relationships between three methods and the beta-quantification-determined LDL-C values[26,37,38]: r=0.985,[38] r=0.983,[26] R^2=0.807[37] for Friedewald; r=0.981,[38] 0.987[26] for Martin/Hopkins; r=0.985,[38] R^2=0.889[37] for NIH equation 2.

Our study found good concordance between Friedewald and Martin/Hopkins, as well as Friedewald and NIH equation 2 methods in classifying individuals into general guideline LDL-C category. While the Friedewald equation tends to underestimate LDL-C, results from Dinç Asarcıklı et al.[42] supported that Friedewald, Martin/Hopkins, and NIH equation 2 was able to correctly identify 96.9%–98.1% of patients. In present study when comparing Friedewald and Martin/Hopkins, the Friedewald classified 93.0%, 3.1%, and 4.0% of the sample into the same (i.e., tied), one-level higher, or one-level lower than the Martin/Hopkins classifications. when comparing Friedewald and NIH equation 2, the Friedewald classified 95.4%, 0.1%, and 4.5% of the sample into the same (i.e., tied), one-level higher, or one-level lower than the NIH equation 2 classifications.

Nowadays, the most accurate method to calculate LDL-C is still a topic of debate among researchers and clinicians. Some are continuing to investigate which equation is superior to others, while others recommend taking into account the level of TGs when selecting an equation.[44] It may be advantageous to suggest that the current healthcare system should include LDL-C values estimated from all three methods: Friedewald, Martin/Hopkins, and NIH equation 2, in the electronic medical database. The accuracy of LDL-C estimation methods varies depending on the patient's TG levels, and the computation of all three LDL-C estimates is comparatively simple to administer. By having all three methods available, providers can select the method that best suits the patient's needs. Further research is necessary to discover more accurate, timely, and cost-effective methods for measuring LDL-C levels.

This study involves several limitations. Firstly, the use of secondary data sources means that the researchers did not have control over the data collection procedures. The National Health and Nutrition Examination Survey (NHANES) employs several quality assurance and quality control protocols, which meet the 1988 Clinical Laboratory Improvement Act mandates, to ensure the accuracy of analyses performed by contract laboratories. Secondly, the analysis was restricted by the available LDL data as TGs were only measured for individuals aged 12 years and older who fasted. It is unknown whether sampling bias existed between those who had LDL estimates and those who did not. Thirdly, we did not compare directly measured LDL cholesterol values with the values obtained by the respective formulas.

Lastly, the comparison between the Friedewald equation and the two alternative equations for estimating LDL was conducted without subgroup analyses. Future studies should aim to compare the performance and agreements of the three equations with demographic variables, such as age group, sex, race/ethnicity, and other associated socioeconomic factors.

Understanding the practical differences in LDL-C calculations can be helpful in facilitating decision-making during a paradigm shift. The continuous evolution of novel therapeutics and treatment targets necessitates the redefinition of LDL-C estimation methods, and as such, further research is needed. Presenting LDL-C calculations from all three methods in current healthcare practices can potentially yield benefits.

SUPPLEMENTARY MATERIAL

Supplementary Table 1
Baseline characteristics of participants

Click here to view

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