# Underestimation of LDL Cholesterol Below 70 mg/dL (1.8 mmol/L) by Friedewald, Hopkins and a "Direct" Assay Compared to Preparative Ultracentrifugation Leading to the Overestimation of LDL Cholesterol Reduction for New Drugs in Development.

*Traci Turner MD*, Nan Plunkett BS*, Rong Zhou PhD**, Miriam Zangmeister MS**, Christine Fritz MS*, Matt Kelso PhD PharmD*, Evan A. Stein MD PhD**

## Abstract

**Background:** Calculated LDL cholesterol (LDL-C) by the Friedewald formula (LDL-$C_F$) has been the basis for clinical and regulatory decision making for >40 years. As clinical guidelines and new therapeutic agents reduce LDL-C levels to below levels originally validated by LDL-$C_F$, studies show substantial underestimation and an alternative formula, Hopkins (LDL-$C_H$), or "direct/homogeneous" (LDL-$C_D$) assay has been proposed. We compare LDL-C by these methods to the "gold standard," preparative ultracentrifugation (LDL-$C_P$), in 1,299 samples with 961 ≤70 mg/dL.

**Methods:** Patient samples were analyzed in a central laboratory that was CDC-NHLBI Part 3 Standardized for lipid measurements. LDL-$C_F$, LDL-$C_H$, LDL-$C_D$, and LDL-$C_P$ were compared including at clinically important cut points of 100, 70, 50, and 25 mg/dL and within each cut point by triglyceride (TG) levels.

**Results:** See tables. While the difference between LDL-C methods were significant at nearly all levels, the differences increased and became clinically meaningful as LDL-C decreased <70 mg/dL and further deteriorated ≤50 and 25 mg/dL, especially for LDL-$C_F$ and LDL-$C_H$. Below 70 mg/dL, for each 100 mg/dL TG increase >100 mg/dL these differences increased irrespective of LDL-C method.

**Conclusion:** Traditional or novel formulas for calculating LDL-C, and "direct" LDL-C measurement show significant and clinically meaningful differences when true LDL-C is <70 mg/dL; and even moderate TG increases have major consequences. As measurements of LDL-C by these commonly used methods underestimates lower LDL-C they result in substantial underestimation of post-treatment LDL-C, which in turn overestimates the reduction in LDL-C with new more effective agents.

## Introduction

- LDL-C is a well-established causative and surrogate biomarker for the development and progression of atherosclerosis.[1,2]
- Large, prospective clinical trials, with statins and recently ezetimibe added to statins, have demonstrated LDL-C lowering to be directly related to risk reduction of morbidity and mortality in cardiovascular (CV) events.[3-6]
- LDL-C, determined by the Friedewald formula (LDL-$C_F$), has been used to calculate LDL-C for the last 4 decades, and is cost-effective and widely accepted by regulatory and guideline committees when TG is <400 mg/dL.[7]
- LDL-C lowering agents in development, such as PCSK-9 (proprotein convertase subtilisin/kexin type 9) inhibitors, have the ability to achieve very low LDL-C, often well below 50 mg/dL.[8-9] While the Friedewald formula was originally validated in patients with LDL-C >70 mg/dL, and has proven robust and reliable for patients with LDL-C above this level, its accuracy and validity for lower LDL-C levels has recently been questioned.[10-11]
- An alternative formula, the "Hopkins" or "novel" formula, has been proposed. This formula was derived against a method known as VAP, which was in turn validated against the "gold standard" preparative ultracentrifugation (PUC), using a variable TG:VLDL-C ratio (varying from 3.1 to 11.9) dependent on total cholesterol (TC), TG, and non–HDL-C levels.[12]
- The Hopkins formula has not been compared to LDL-C determined by preparative PUC.
- LDL-$C_D$, or homogenous methods, are detergent based assays which are based on inhibition of measurement of cholesterol in other lipoproteins from being measured, and were originally introduced to measure LDL-C where TG >400 mg/dL or patients were non-fasting. However their performance vary by manufacturer and from reagent generation within the same manufacturer. Their accuracy relative to PUC has also been shown to deteriorate in diseased (primarily dyslipidemic and cardiovascular) populations. There is also no data on their accuracy at low LDL-C concentrations.[13]
- We report the results of LDL-C measured by the "gold standard" method, PUC, as compared to LDL-C estimated by the Friedewald and Hopkins formulas and "directly" measured using a homogenous assay in 1299 samples including 961 with LDL-C ≤70 mg/dL and 896 ≤50 mg/dL.

## Methodology

### *Samples*

- Serum or plasma samples were collected after an overnight fast (water only) and analyzed for TC, TG, high density lipoprotein cholesterol (HDL-C), LDL-$C_P$, and LDL-$C_D$ and were evaluated for those with TG ≤400 mg/dL, resulting in total of 1299 comparisons.
- The samples were from either patients in a specialized lipid clinic or participants in clinical trials, and included pediatric patient samples. All samples were received de-identified of demographic information.

### *Analytical Methods*

- TC, the cholesterol content of isolated fractions, and TG were measured in the central laboratory (Medpace Reference Laboratories, Cincinnati, US), which maintained CDC-NHLBI Lipid Standardization Program Part III throughout the period (Participant number LSP-395).[14]
- Analysis of cholesterol and TG was by enzymatic methods on a Beckman Coulter AU Series automated chemistry analyzer with in-house developed serum calibrators directly traceable to CDC-NHLBI reference procedures.[14]
- LDL-$C_P$ was performed using the method modified from the Lipid Research Clinics methods manual.[15] Serum or plasma was overlaid with normal saline (density 1.006 g/mL) and centrifuged (Beckman Ultracentrifuge Model # L-90K and rotor, Type 50.4) at 40,000 rpm for 18-22 hours at 10°C to separate very low-density lipoprotein (VLDL) in the supernatant ("top" fraction) from LDL and HDL in the infranatant or "bottom" fraction. The cholesterol concentration of the infranatant was measured. All apolipoprotein B-containing lipoproteins, VLDL, intermediate density lipoprotein (IDL), LDL, and Lp(a) were precipitated from serum using 50 kDa dextran sulfate with magnesium ions ($MgCl_2$),[16] and the cholesterol in the remaining HDL fraction was measured. The HDL-C concentration was subtracted from the infranatant cholesterol to provide the PUC LDL-C value. VLDL-C was calculated by subtracting the "bottom" fraction cholesterol from TC. The ratio of cholesterol in VLDL to TG was calculated by VLDL-C/TG.
- Calculated LDL-C was estimated from the Friedewald formula[7] where: LDL-$C_F$ = TC – (HDL-C + TG/5) [for mmol/L, LDL-C = TC – HDL-C – (TG/2.2)] and from the Hopkins formula where: LDL-$C_H$ = TC – (HDL-C + TG/ adjustable factor mg/dL); the adjustable factor was determined as the strata-specific median TG:VLDL-C ratio.[12]
- LDL-$C_D$ was measured by a homogeneous enzymatic assay using Roche C.f.a.s. Lipid Calibrator and LDL-C plus 2nd generation reagent (both traceable to the Cholesterol Reference Method Laboratory Network accuracy base for LDL-C) on a Beckman Coulter AU Series automated chemistry analyzer.

### *Statistical Methods*

- Summary statistics, mean (standard deviation [SD]) values for continuous variables, and numbers of patients and percentages for categorical variables were calculated on measured and calculated lipid parameters.
- Subgroup analyses based on the differences between LDL-$C_F$, LDL-$C_H$, LDL-$C_D$ as compared to LDL-$C_P$ for each sample were performed based on LDL-$C_F$ and TG levels at selected cut points. Similar analysis was done for VLDL-C/TG ratio.
- The percent difference of LDL-$C_H$ from LDL-$C_P$ was presented graphically in an Altman-Bland plot.

*Figure 1: Altman-Bland Plot LDL-C by Friedewald and Preparative Ultracentrifugation*

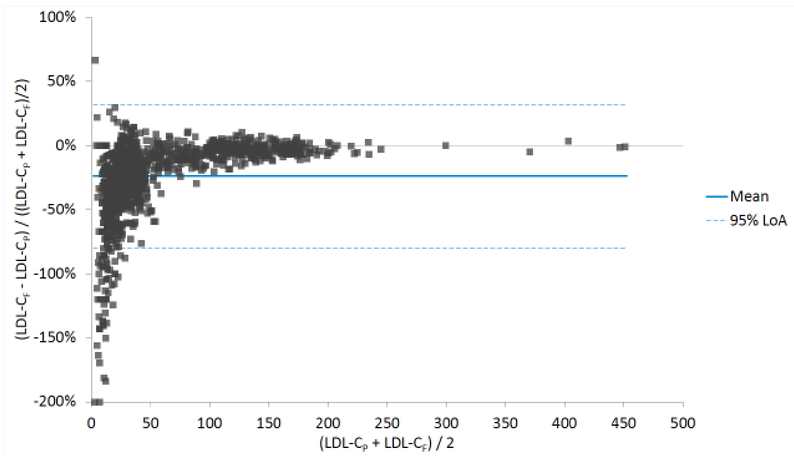



*Figure 2: Altman-Bland Plot LDL-C by Hopkins and Preparative Ultracentrifugation*

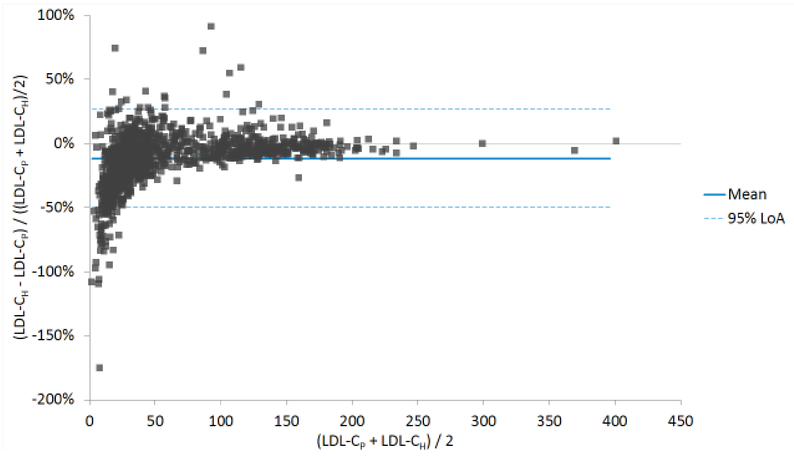
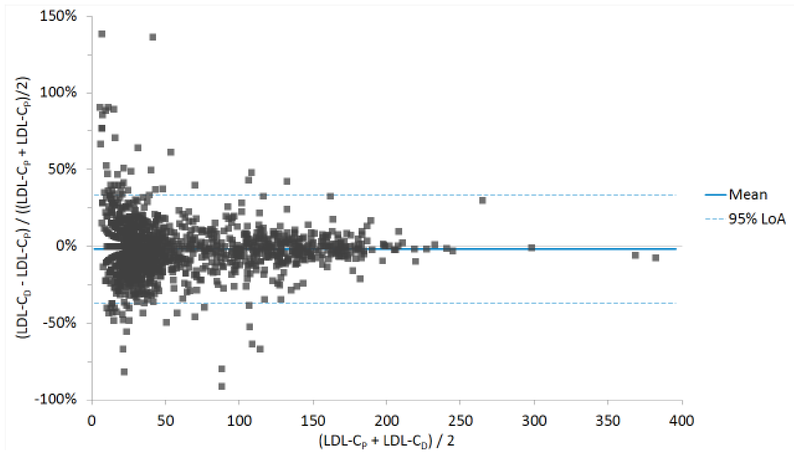



*Figure 3: Altman-Bland Plot LDL-C by Direct Method and Preparative Ultracentrifugation*




## Results

- Overall results for the 1,299 samples are shown in Table 1. LDL-C ranged from 2 to 453 mg/dL by PUC, 0 to 449 mg/dL by Friedewald (overall mean [±SD] % difference -18.9 ±19.34), 1 to 446 mg/dL by Hopkins (overall mean [±SD] % difference -9.3 ±17.83), and 7 to 369 mg/dL by the direct method (overall mean [±SD] % difference -0.8 ±21.91). TG ranged from 28 to 394 mg/dL.
- Assessment based on selected calculated LDL-C cut points (Table 2) resulted in 947 results ≤70 mg/dL, 860 ≤50 mg/dL, and 322 ≤25 mg/dL.

**Table 1: Summary Statistics for Lipid Parameters**

| Lipid Parameter (units) | N | Mean | SD | Min | Max |
|---|---|---|---|---|---|
| TC (mg/dL) | 1299 | 126.7 | 57.10 | 51 | 515 |
| HDL-C (mg/dL) | 1299 | 48.9 | 14.17 | 18 | 124 |
| TG (mg/dL) | 1299 | 123.6 | 64.98 | 28 | 394 |
| Calculated LDL-$C_F$ by Friedewald (mg/dL) | 1299 | 53.1 | 53.53 | 0 | 449 |
| Calculated LDL-$C_H$ by Hopkins (mg/dL) | 1299 | 56.6 | 53.06 | 1 | 446 |
| LDL-$C_P$ by preparative ultracentrifugation (mg/dL) | 1299 | 59.5 | 52.67 | 2 | 453 |
| "Direct" LDL-$C_D$ (mg/dL) | 1289 | 57.1 | 49.00 | 7 | 369 |
| % Difference Friedewald[a] | 1299 | -18.9 | 19.34 | -100 | 100 |
| % Difference Hopkins[b] | 1299 | -9.3 | 17.83 | -90 | 150 |
| % Difference "Direct"[c] | 1289 | -0.8 | 21.91 | -63 | 450 |
| VLDL-C[d] (mg/dL) | 1299 | 18.3 | 11.48 | 2 | 73 |
| VLDL-C/TG | 1299 | 0.146 | 0.0434 | 0.028 | 0.433 |

a % difference = 100*( LDL-$C_F$ – LDL-$C_P$)/ LDL-$C_P$
b % difference = 100*(LDL-$C_H$ – LDL-$C_P$)/ LDL-$C_P$
c % difference = 100*(LDL-$C_D$ – LDL-$C_P$)/LDL-$C_P$
d VLDL-C = TC – HDL-C – LDL-$C_P$

**Table 2: Summary Statistics of LDL-$C_D$, LDL-$C_F$, and LDL-$C_H$ and LDL-$C_P$ by LDL-$C_P$ Categories**

| LDL-$C_P$ (mg/dL) | N | LDL-$C_P$ (mg/dL) Mean (SD) | LDL-$C_D$ (mg/dL) Mean (SD) | % Difference[a] Mean (SD) | % Difference[a] p-value | LDL-$C_F$ (mg/dL) Mean (SD) | % Difference[b] Mean (SD) | % Difference[b] p-value | LDL-$C_H$ (mg/dL) Mean (SD) | % Difference[c] Mean (SD) | % Difference[c] p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ≤25 | 322 | 18.1 (4.85) | 18.9 (5.17)(N=319) | 8.8 (37.08) | <.0001 | 12.3 (5.67) | -32.9 (24.75) | <.0001 | 14.6 (5.88) | -19.7 (24.61) | <.0001 |
| 26-50 | 538 | 36.0 (6.65) | 34.3 (7.33)(N=535) | -4.3 (13.26) | <.0001 | 28.5 (7.25) | -20.9 (14.69) | <.0001 | 32.8 (8.37) | -9.3 (15.60) | <.0001 |
| 51-70 | 87 | 59.5 (6.08) | 57.0 (8.65)(N=87) | -4.1 (11.71) | 0.0016 | 50.9 (9.88) | -14.3 (14.25) | <.0001 | 58.1 (9.29) | -2.3 (12.58) | 0.0875 |
| 71-100 | 76 | 86.2 (8.78) | 83.6 (11.55) (N=74) | -2.7 (10.18) | 0.0253 | 80.2 (9.88) | -6.9 (6.40) | <.0001 | 84.2 (10.08) | -2.2 (8.56) | 0.0317 |
| 101-200 | 258 | 138.0 (24.86) | 132.9 (28.14)(N=258) | -3.7 (10.46) | <.0001 | 133.4 (25.10) | -3.4 (5.13) | <.0001 | 135.8 (24.33) | -1.4 (5.71) | 0.0001 |
| >200 | 18 | 267.5 (88.18) | 235.6 (57.59)(N=16) | -3.5 (5.34) | 0.0190 | 261.9 (90.02) | -2.4 (3.15) | 0.0051 | 261.4 (88.37) | -2.4 (2.83) | 0.0019 |
| ≤50 | 860 | 29.3 (10.57) | 28.6 (9.99)(N=854) | 0.6 (25.75) | 0.5097 | 22.4 (10.34) | -25.4 (19.94) | <.0001 | 26.0 (11.57) | -13.2 (20.10) | <.0001 |
| ≤70 | 947 | 32.1 (13.44) | 31.2 (12.85)(N=941) | 0.1 (24.82) | 0.8546 | 25.1 (13.18) | -24.4 (19.74) | <.0001 | 28.9 (14.68) | -12.2 (19.78) | <.0001 |
| ≤100 | 1023 | 36.1 (19.34) | 35.0 (18.67)(N=1015) | -0.1 (24.06) | 0.9371 | 29.2 (19.43) | -23.1 (19.62) | <.0001 | 33.0 (20.42) | -11.4 (19.35) | <.0001 |

a % difference = 100*( LDL-$C_D$ – LDL-CP)/ LDL-$C_P$
b % difference = 100*(LDL-$C_F$ – LDL-CP)/ LDL-$C_P$
c % difference = 100*(LDL-$C_H$ – LDL-CP)/LDL-$C_P$
p-values are from a one sample t-test performed on % difference

***Note:*** *Overall N=1289 for direct LDL and N=1299 for other parameters.*

- LDL-$C_F$ using the Friedewald formula underestimated LDL-C as compared to LDL-$C_P$ at all LDL-C cut-points. LDL-$C_F$ showed a minimal difference of -3.4% when LDL-C was between 101-200 mg/dL). However, as values decreased below 100 mg/dL Friedewald underestimated LDL-C compared to PUC by 6.9% between 100 and 71 mg/dL, 14.3% between 70 and 51 mg/dL, 20.9% between 50 and 26 mg/dL, and 32.9% at 25 mg/dL or below. Within each LDL-C cut-point the difference between Friedewald and PUC increases for every 100 mg/dL rise in TG, especially at LDL-C below 50 and 25 mg/dL. Analysis within each LDL-C cut point by each 100 mg/dL increase of TG (i.e., ≤100, 101 ≤200, 201 ≤300, 301 ≤400) is shown in Tables 3 and 4 which show mean percent and mean absolute differences between Friedewald and PUC respectively.

**Table 3: Summary Statistics for *% Difference* of Calculated LDL-$C_F$ by *Friedewald* and LDL-$C_P$**

| LDL-$C_P$ (mg/dL) | TG Level (mg/dL) ≤100 N | Mean (SD) | 101-200 N | Mean (SD) | 201-300 N | Mean (SD) | 301-400 N | Mean (SD) | Overall N | Mean (SD) | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ≤25 | 184 | -22.9 (21.50) | 125 | -43.9 (20.84) | 12 | -66.2 (26.05) | 1 | -95.7 (.) | 322 | -32.9 (24.75) | <.0001 |
| 26-50 | 251 | -12.8 (9.97) | 228 | -26.2 (12.37) | 53 | -34.9 (19.27) | 6 | -35.9 (21.78) | 538 | -20.9 (14.69) | <.0001 |
| 51-70 | 42 | -6.7 (5.87) | 21 | -12.5 (9.70) | 16 | -27.4 (13.56) | 8 | -32.9 (22.00) | 87 | -14.3 (14.25) | <.0001 |
| 71-100 | 33 | -4.8 (5.34) | 29 | -7.3 (6.67) | 12 | -11.2 (6.26) | 2 | -9.4 (9.63) | 76 | -6.9 (6.40) | <.0001 |
| 101-200 | 88 | -2.3 (3.26) | 114 | -2.9 (4.44) | 42 | -6.2 (6.95) | 14 | -5.1 (9.38) | 258 | -3.4 (5.13) | <.0001 |
| >200 | 9 | -1.7 (2.92) | 5 | -2.3 (3.64) | 3 | -3.0 (3.06) | 1 | -7.2 (.) | 18 | -2.4 (3.15) | 0.0051 |
| Overall | 607 | -13.3 (15.52) | 522 | -23.5 (20.00) | 138 | -25.3 (23.58) | 32 | -21.0 (24.77) | 1299 | -18.9 (19.34) | <.0001 |
| p-value | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | | |

***Note:*** % difference = 100*(calculated LDL-$C_F$ – LDL-$C_P$)/LDL-$C_P$
P-values are from a one sample t-test performed on % difference.

**Table 4: Summary Statistics for *Difference* of Calculated LDL-$C_F$ by *Friedewald* and LDL-$C_P$**

| LDL-$C_P$ (mg/dL) | TG Level (mg/dL) ≤100 N | Mean (SD) | 101-200 N | Mean (SD) | 201-300 N | Mean (SD) | 301-400 N | Mean (SD) | Overall N | Mean (SD) | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ≤25 | 184 | -4.0 (3.00) | 125 | -7.8 (3.64) | 12 | -13.0 (4.53) | 1 | -22.0 (NC) | 322 | -5.8 (4.15) | <.0001 |
| 26-50 | 251 | -4.6 (3.42) | 228 | -9.3 (4.14) | 53 | -13.2 (6.88) | 6 | -12.7 (6.71) | 538 | -7.5 (5.14) | <.0001 |
| 51-70 | 42 | -3.9 (3.44) | 21 | -7.6 (5.57) | 16 | -15.9 (7.92) | 8 | -20.6 (13.94) | 87 | -8.5 (8.61) | <.0001 |
| 71-100 | 33 | -4.2 (4.40) | 29 | -6.2 (5.67) | 12 | -9.8 (5.36) | 2 | -7.5 (7.78) | 76 | -5.9 (5.39) | <.0001 |
| 101-200 | 88 | -3.1 (4.47) | 114 | -4.1 (6.11) | 42 | -8.4 (8.77) | 14 | -6.8 (11.38) | 258 | -4.6 (6.74) | <.0001 |
| >200 | 9 | -4.3 (8.94) | 5 | -5.0 (8.40) | 3 | -7.0 (7.00) | 1 | -15.0 (NC) | 18 | -5.6 (8.15) | 0.0102 |
| Overall | 607 | -4.1 (3.68) | 522 | -7.5 (5.12) | 138 | -11.6 (7.73) | 32 | -12.1 (11.99) | 1299 | -6.5 (5.76) | <.0001 |
| p-value | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | | |

***Note:*** % difference = 100*(calculated LDL-$C_F$ – LDL-$C_P$)/LDL-$C_P$
P-values are from a one sample t-test performed on % difference.

- Overall, LDL-$C_H$ derived from the Hopkins method underestimated LDL-C as compared to PUC at all LDL-C cut points, though not to the same degree as LDL-C estimated by Friedewald. The underestimation using LDL-$C_H$ increased as LDL-C levels decreased; 2.2% between 100 and 71 mg/dL, 2.3% between 70 and 51 mg/dL, 9.3% between 50 and 26 mg/dL, and 19.7% at 25 mg/dL or below. For TG levels ≤200 mg/dL, Hopkins underestimated LDL-C at all LDL-C cut points (overall mean difference 15.5% for TG ≤100 mg/dL, 8.2% for TG 101 to 200 mg/dL) and overestimated LDL-C when TG levels were ≥201 mg/dL (overall mean difference 6.6% for TG 201 to 300 mg/dL, 20.3% for TG 301 to 400 mg/dL), shown in Tables 5 and 6.

**Table 5: Summary Statistics for *% Difference* of Calculated LDL-$C_H$ by *Hopkins* and LDL-$C_P$**

| LDL-$C_P$ (mg/dL) | TG Level (mg/dL) ≤100 N | Mean (SD) | 101-200 N | Mean (SD) | 201-300 N | Mean (SD) | 301-400 N | Mean (SD) | Overall N | Mean (SD) | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ≤25 | 184 | -26.2 (24.50) | 125 | -14.1 (19.94) | 12 | 15.5 (20.91) | 1 | 43.5 (.) | 322 | -19.7 (24.61) | <.0001 |
| 26-50 | 251 | -14.6 (9.79) | 228 | -9.8 (12.02) | 53 | 11.1 (18.81) | 6 | 53.3 (24.55) | 538 | -9.3 (15.60) | <.0001 |
| 51-70 | 42 | -7.8 (6.55) | 21 | -2.6 (9.56) | 16 | 3.2 (11.64) | 8 | 15.9 (22.59) | 87 | -2.3 (12.58) | 0.0875 |
| 71-100 | 33 | -6.4 (5.54) | 29 | -2.1 (7.02) | 12 | 5.6 (8.04) | 2 | 19.5 (13.40) | 76 | -2.2 (8.56) | 0.0317 |
| 101-200 | 88 | -3.9 (3.16) | 114 | -1.4 (4.87) | 42 | 0.5 (5.85) | 14 | 8.8 (9.79) | 258 | -1.4 (5.71) | 0.0001 |
| >200 | 9 | -2.7 (2.82) | 5 | -2.5 (3.68) | 3 | -1.2 (2.49) | 1 | -2.9 (.) | 18 | -2.4 (2.83) | 0.0019 |
| Overall | 607 | -15.5 (17.10) | 522 | -8.2 (13.86) | 138 | 6.6 (15.12) | 32 | 20.3 (23.84) | 1299 | -9.3 (17.83) | <.0001 |
| p-value | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | | |

***Note:*** % difference = 100*(calculated LDL-$C_H$ – LDL-$C_P$)/LDL-$C_P$
P-values are from a one sample t-test performed on % difference.

**Table 6: Summary Statistics for *Difference* of Calculated LDL-$C_H$ by *Hopkins* and LDL-$C_P$**

| LDL-$C_P$ (mg/dL) | TG Level (mg/dL) ≤100 N | Mean (SD) | 101-200 N | Mean (SD) | 201-300 N | Mean (SD) | 301-400 N | Mean (SD) | Overall N | Mean (SD) | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ≤25 | 184 | -4.6 (3.17) | 125 | -2.6 (3.50) | 12 | 3.1 (4.42) | 1 | 10.0 (NC) | 322 | -3.5 (3.78) | <.0001 |
| 26-50 | 251 | -5.2 (3.33) | 228 | -3.5 (4.01) | 53 | 4.1 (6.99) | 6 | 18.7 (5.82) | 538 | -3.3 (5.43) | <.0001 |
| 51-70 | 42 | -4.5 (3.79) | 21 | -1.4 (5.65) | 16 | 1.8 (6.65) | 8 | 8.8 (12.69) | 87 | -1.4 (7.17) | 0.0761 |
| 71-100 | 33 | -5.6 (4.58) | 29 | -1.8 (6.05) | 12 | 4.6 (6.61) | 2 | 15.0 (9.90) | 76 | -2.0 (7.11) | 0.0173 |
| 101-200 | 88 | -5.2 (4.30) | 114 | -2.2 (6.63) | 42 | 0.3 (7.69) | 14 | 10.2 (11.44) | 258 | -2.2 (7.36) | <.0001 |
| >200 | 9 | -7.3 (8.35) | 5 | -5.6 (8.56) | 3 | -3.0 (5.57) | 1 | -6.0 (NC) | 18 | -6.1 (7.50) | 0.0032 |
| Overall | 607 | -5.0 (3.67) | 522 | -2.8 (4.87) | 138 | 2.5 (7.09) | 32 | 11.2 (11.10) | 1299 | -2.9 (5.84) | <.0001 |
| p-value | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | | |

***Note:*** % difference = 100*(calculated LDL-$C_H$ – LDL-$C_P$)/LDL-$C_P$
P-values are from a one sample t-test performed on % difference.

- As compared to PUC, LDL-C measured with the "direct" method was accurate overall with a % difference of -0.8 (p-value 0.1699). However, the differences at all LDL-C cut-points were statistically significant with underestimation of LDL-C as compared to PUC; 3.7% between 101 and 200 mg/dL, 2.7% between 100 and 71 mg/dL, 4.1% between 70 and 51 mg/dL, and 4.3% between 50 and 26 mg/dL. When LDL-C was ≤25 mg/dL, the direct method overestimated LDL-C by 8.8% (Tables 7 and 8).

**Table 7: Summary Statistics for *% Difference* of Calculated LDL-$C_D$ by *Direct Method* and LDL-$C_P$**

| LDL-$C_P$ (mg/dL) | TG Level (mg/dL) ≤100 N | Mean (SD) | 101-200 N | Mean (SD) | 201-300 N | Mean (SD) | 301-400 N | Mean (SD) | Overall N | Mean (SD) | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ≤25 | 182 | 7.5 (41.56) | 124 | 10.1 (30.07) | 12 | 16.4 (33.32) | 1 | 0.0 (N/A) | 319 | 8.8 (37.08) | <.0001 |
| 26-50 | 250 | -5.9 (12.20) | 227 | -4.8 (13.52) | 52 | 3.7 (13.44) | 6 | 12.9 (9.79) | 535 | -4.3 (13.26) | <.0001 |
| 51-70 | 42 | -4.9 (9.85) | 21 | -1.8 (13.26) | 16 | -5.5 (12.13) | 8 | -3.1 (16.52) | 87 | -4.1 (11.71) | 0.0016 |
| 71-100 | 32 | -5.3 (8.68) | 28 | -3.1 (11.03) | 12 | 4.1 (9.85) | 2 | 3.3 (6.47) | 74 | -2.7 (10.18) | 0.0253 |
| 101-200 | 88 | -5.2 (8.63) | 114 | -3.5 (9.94) | 42 | -3.6 (14.30) | 14 | 3.2 (9.56) | 258 | -3.7 (10.46) | <.0001 |
| >200 | 7 | -4.7 (3.81) | 5 | -4.3 (8.26) | 3 | -0.7 (2.76) | 1 | 0.5 (N/A) | 16 | -3.5 (5.34) | 0.0190 |
| Overall | 601 | -1.6 (25.31) | 519 | -0.8 (19.16) | 137 | 1.4 (16.71) | 32 | 3.2 (12.08) | 1289 | -0.8 (21.91) | 0.1699 |
| p-value | | 0.1130 | | 0.3644 | | 0.3168 | | 0.1386 | | | |

***Note:*** % difference = 100*(calculated LDL-$C_D$ – LDL-$C_P$)/LDL-$C_P$
P-values are from a one sample t-test performed on % difference.

**Table 8: Summary Statistics for *Difference* of Calculated LDL-$C_D$ by *Direct Method* and LDL-$C_P$**

| LDL-$C_P$ (mg/dL) | TG Level (mg/dL) ≤100 N | Mean (SD) | 101-200 N | Mean (SD) | 201-300 N | Mean (SD) | 301-400 N | Mean (SD) | Overall N | Mean (SD) | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ≤25 | 182 | 0.4 (3.51) | 124 | 1.1 (3.43) | 12 | 3.3 (6.80) | 1 | 0.0 (N/A) | 319 | 0.8 (3.68) | 0.0002 |
| 26-50 | 250 | -2.2 (4.26) | 227 | -1.9 (4.71) | 52 | 1.2 (5.13) | 6 | 4.5 (3.21) | 535 | -1.7 (4.68) | <.0001 |
| 51-70 | 42 | -2.9 (6.01) | 21 | -1.2 (8.38) | 16 | -3.2 (6.91) | 8 | -2.5 (9.96) | 87 | -2.5 (7.11) | 0.0015 |
| 71-100 | 32 | -4.5 (7.34) | 28 | -2.7 (9.25) | 12 | 3.1 (8.61) | 2 | 2.5 (4.95) | 74 | -2.4 (8.58) | 0.0178 |
| 101-200 | 88 | -6.8 (10.75) | 114 | -5.0 (13.73) | 42 | -4.8 (20.06) | 14 | 3.1 (11.78) | 258 | -5.1 (14.07) | <.0001 |
| >200 | 7 | -13.3 (11.00) | 5 | -8.8 (16.54) | 3 | -2.0 (6.24) | 1 | 1.0 (N/A) | 16 | -8.9 (12.31) | 0.0114 |
| Overall | 601 | -2.4 (6.43) | 519 | -1.9 (8.22) | 137 | -0.9 (12.52) | 32 | 1.8 (9.50) | 1289 | -1.9 (8.11) | <.0001 |
| p-value | | <.0001 | | <.0001 | | 0.4182 | | 0.2968 | | | |

***Note:*** % difference = 100*(calculated LDL-$C_D$ – LDL-$C_P$)/LDL-$C_P$
P-values are from a one sample t-test performed on % difference.

## Conclusions

- LDL-C, as determined by both the Friedewald and Hopkins formulas, and "direct" method, underestimates LDL-C as compared to PUC at pre-specified LDL-C cut-points. For the estimating equations, the underestimation becomes more pronounced and increasingly clinically significant as LDL-C decreases below 100 mg/dL.
- For LDL-C ≤100 mg/dL, each 100 mg/dL rise in TG results in increasing underestimation of LDL-C as determined by Friedewald, with an average difference of 39.2% when LDL-C is ≤25 mg/dL, increasing to >65% when TG levels are >200 mg/dL.
- The Hopkins formula also underestimates LDL-C as compared to PUC when TG levels are ≤200 mg/dL, but overestimates LDL-C when TG are >200 mg/dL.
- Overall, the "direct" homogenous method for measuring LDL-C was more reliable and did not show increasing differences with various TG cut points.
- For drugs in development, accurate measurement of key efficacy parameters, such as LDL-C, is of paramount importance to assess response to drug. If LDL-C is underestimated post-treatment, it results in overestimation of both the percent and absolute reductions achieved by the drug.
- For cardiovascular disease (CVD) outcome trials of LDL-C reducing agents where LDL-C is reduced to low levels, it is critical to have accurate assessment of absolute LDL-C reductions as the absolute reduction in LDL-C (not % decrease) which relates to and predicts, reduction in CVD events.
- In clinical practice, under or overestimation of LDL-C levels can lead to erroneous treatment decisions, such as under treatment of patients at high risk of CVD.

*Figure 4: % Difference (mean ± SE) in LDL-C (Friedewald)*

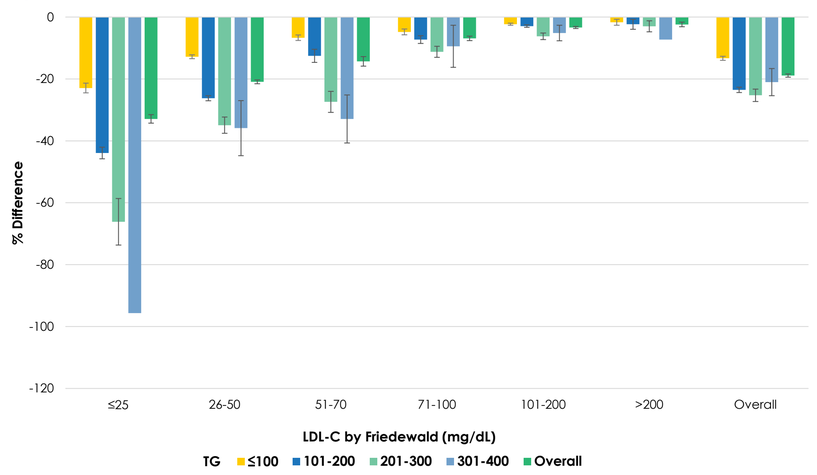



*Figure 5: % Difference (mean ± SE) in LDL-C (Hopkins)*

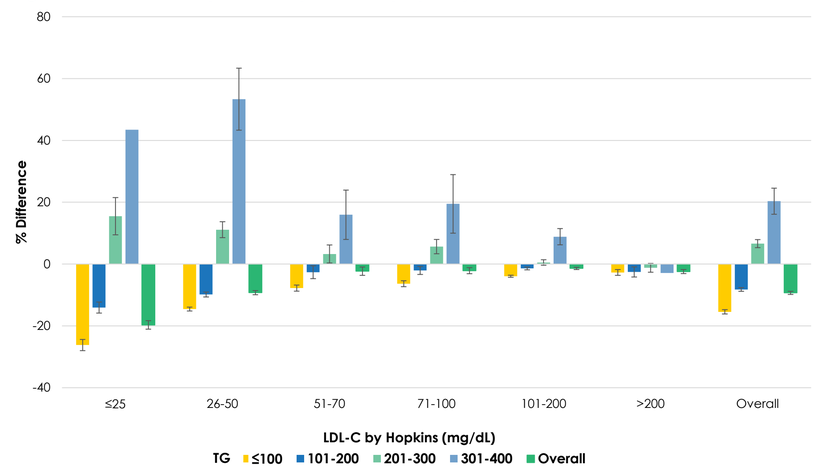
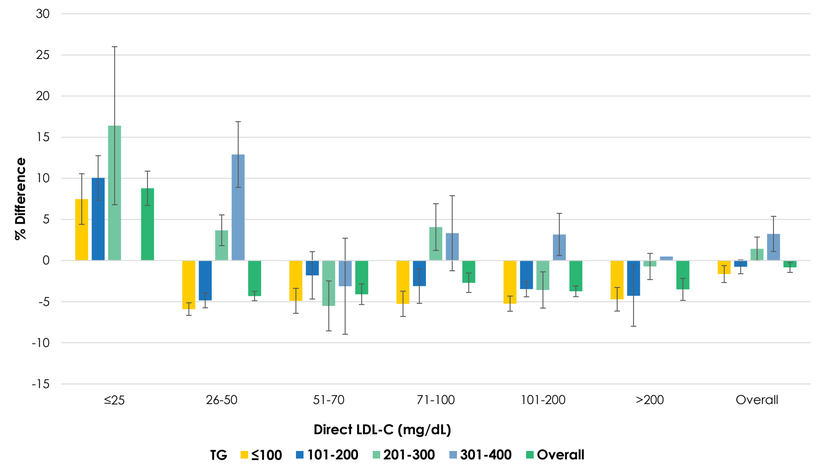



*Figure 6: % Difference (mean ± SE) in LDL-C (Direct)*

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*Medpace Reference Laboratories, Cincinnati, Ohio, US
**Medpace Biostatistics, Cincinnati, Ohio, US

Traci Turner vCard

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