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Fasting vs Non-Fasting and Low-Density Lipoprotein-Cholesterol Accuracy

Running Title: *Sathiyakumar et al.; Fasting vs Non-Fasting LDL-C*

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Abstract

Background—Recent recommendations favoring non-fasting lipid assessment may impact low-density lipoprotein-cholesterol (LDL-C) estimation. The novel method of LDL-C estimation (LDL-C_N) uses a flexible approach to derive patient-specific triglyceride (TG) to very low-density lipoprotein-cholesterol ratios. This adaptability may confer an accuracy advantage in non-fasting patients over the fixed approach of the classical Friedewald method (LDL-C_F).
Methods—We used a US cross-sectional sample of 1,545,634 patients (959,153 fasting ≥10-12 hours; 586,481 non-fasting) from the second harvest of the Very Large Database of Lipids study to assess for the first time the impact of fasting status on novel LDL-C accuracy. Rapid ultracentrifugation was used to directly measure LDL cholesterol content (LDL-C_D). Accuracy was defined as the percentage of LDL-C_D falling within an estimated LDL-C (LDL-C_N or LDL-C_F) category by clinical cutpoints. For low estimated LDL-C (<70 mg/dL), we evaluated accuracy by TG levels. The magnitude of absolute and percent differences between LDL-C_D and estimated LDL-C (LDL-C_N or LDL-C_F) was stratified by LDL-C and TG categories.
Results—In both fasting and non-fasting samples, accuracy was higher with the novel method across all clinical LDL-C categories (range: 87-94%) compared to Friedewald estimation (range: 71-93%) ($p \leq 0.001$). With LDL-C <70 mg/dL, non-fasting LDL-C_N accuracy (92%) was superior to LDL-C_F (71%) ($p<0.001$). In this LDL-C range, 19% of fasting and 30% of non-fasting patients had differences ≥10 mg/dL between LDL-C_F and LDL-C_D, whereas only 2% and 3% of patients respectively had similar differences with novel estimation. Accuracy of LDL-C <70 mg/dL further decreased as TG increased, particularly for Friedewald estimation (range: 37-96%) versus the novel method (range: 82-94%). With TG 200-399 mg/dL in non-fasting patients, LDL-C_N <70 mg/dL accuracy (82%) was superior to LDL-C_F (37%) ($p<0.001$). In this TG range, 73% of fasting and 81% of non-fasting patients had ≥10 mg/dL differences between LDL-C_F and LDL-C_D, compared to 25% and 20% of patients respectively with LDL-C_N.
Conclusions—Novel adaptable LDL-C estimation performs better in non-fasting samples than the fixed Friedewald estimation, with a particular accuracy advantage in settings of low LDL-C and high TG. In addition to stimulating further study, these results may have immediate relevance to guideline committees, laboratory leadership, clinicians and patients.
Clinical Trial Registration—URL: clinicaltrials.gov Unique Identifier: NCT01698489

Key Words: lipids; low-density lipoprotein cholesterol; Friedewald, novel

Clinical Perspective

What is new?

- In this US cross-sectional analysis, we demonstrate for the first time that the novel, adaptable method of LDL-C estimation is more accurate in non-fasting samples compared to the Friedewald equation.
- Both absolute and percent error between novel-estimated LDL-C and directly-measured LDL-C are smaller and less affected by fasting status compared to Friedewald LDL-C, especially in the estimated LDL-C<70 mg/dL category.

What are the clinical implications?

- In making evidence-based decisions about lipid-lowering therapy, clinicians and patients can place greater confidence in LDL-C results from non-fasting samples that are calculated using the novel method of LDL-C estimation compared to the classical Friedewald equation.
- This accuracy is especially important for high-risk patients with secondary LDL-C goals of <70 mg/dL, a common clinical cutpoint used in international guidelines to initiate and titrate lipid-lowering therapies.

Recent expert recommendations have supported non-fasting lipid assessment.[1–6] Practical advantages to using non-fasting measurements include increasing patient convenience as they avoid separate return visits for lab draws, and improving hospital and clinic efficiency as the need to organize resources around mass patient influx in the morning for blood work is prevented.[1] Moreover, non-fasting triglyceride (TG) and non-high-density lipoprotein-cholesterol (HDL-C) levels may improve cardiovascular risk prediction.[7–11] On the other hand, classification of dyslipidemias was historically derived in fasting samples, and cohort studies and clinical trials have traditionally performed fasting assessments.[12-13]

Ultimately, the choice for fasting or non-fasting lipid assessment may depend on how the lipid profile will be used clinically.[14] If the objective is to make data-driven and guideline-supported decisions with respect to whether a patient qualifies for low-density lipoprotein-cholesterol (LDL-C) lowering therapy or whether an on-treatment LDL-C level is optimal, then clinicians may seek to consider the accuracy of fasting vs non-fasting LDL-C measurements.[15] This may be of increasing relevance to clinicians as practice adapts to greater emphasis on precision in delivery of medical care.

Multiple methods exist for LDL-C assessment, but the Friedewald equation (total cholesterol [TC] – HDL-C – TG/5 in mg/dL) has been the *de facto* clinical standard since the 1970s.[16] The equation was derived in the fasting state and uses a fixed ratio of 5:1 between TGs and very low-density lipoprotein-cholesterol (VLDL-C). With fluctuating TG levels and related variation in the TG:VLDL-C ratio in the post-prandial state, Friedewald and colleagues recognized in their original publication that at lower LDL-C levels, even small errors in VLDL-C estimation may result in significant errors in LDL-C estimation.[16] Indeed, we and others have

shown substantial LDL-C underestimation at low LDL-C and high TG levels when applying the Friedewald equation in modern patients.[17–20]

The clinical scenario of low LDL-C and high TG is increasingly common in clinical practice as a result of new efficacious LDL-lowering therapies and epidemics of obesity and diabetes increasing the prevalence of hypertriglyceridemia. Furthermore, VLDL-C metabolism may be altered in the non-fasting state with variable activity of enzymes such as lipoprotein lipase, thereby affecting the TG content of VLDL-C.[21-22] To address the issue of LDL-C accuracy, direct chemical-based LDL-C assays have been developed, but may be affected by fasting status with added expense and have generally not improved on Friedewald estimation.[23-25] In this context, we previously derived a novel method for LDL-C estimation that uses an adjustable TG:VLDL-C ratio based on TG and non-HDL-C levels.[20] This method improved LDL-C estimation at low LDL-C levels and is now being adopted by laboratories including Quest Diagnostics.[26-27] We and others have speculated that the adaptability of the method may offer an accuracy advantage in non-fasting patients over the fixed Friedewald estimation, however this has not been formally evaluated to date.

This is especially important for high-risk patients with secondary prevention LDL-C goals <70 mg/dL, a common lipid cutpoint used in clinical guidelines to both initiate and intensify lipid-lowering therapy.[1,2,15,28-30] We therefore evaluated for the first time the impact of fasting status on LDL-C accuracy estimated using the novel method compared to LDL-C estimated from the Friedewald equation in a large cross-sectional clinical cohort of over 1.5 million US patients. We further evaluated the absolute and percent differences in using the Friedewald and novel equations to estimate LDL-C based on fasting status.

Methods

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results. Study materials are securely housed at Johns Hopkins University and may be made available through remote access after completion of a data use agreement. Interested investigators may visit the VLDL database clinicaltrials.gov site and may contact the VLDL study Publications and Presentations Committee.[31]

Study population

This is the first study to use the second harvest of the Very Large Database of Lipids (VLDL) study and to evaluate the impact of fasting status on novel LDL-C accuracy. The VLDL study has been described in detail previously.[32] The novel method was derived from the first harvest of VLDL patients; this analysis therefore represents patients who were not included in the derivation cohort for the novel equation. When a patient had more than one lipid profile available, we used the first measurement for each participant. We excluded patients with missing age, sex, and fasting status (fasting or non-fasting), or with incomplete lipid values. Because the original Friedewald equation was designed for patients with TG levels <400 mg/dL, we further excluded participants with TG ≥400 mg/dL.[16] There was no age restriction. Supplemental Figure 1 illustrates the patient selection process.

A total of 1,545,634 participants met criteria for analysis, including 959,153 fasting patients and 586,481 non-fasting patients. Our study was declared exempt by the Johns Hopkins Institutional Review Board, which waived the requirement of informed consent as we used only de-identified data routinely collected during clinical lipid determinations. All authors attest to full data access and take responsibility for data integrity and analysis. The VLDL study is registered at clinicaltrials.gov (NCT01698489).

Lipid measurements

Patients were deemed as fasting if the clinician-ordered lipid sample called for 10-12 hours of no oral intake other than water or medications prior to sample collection. Vertical Auto Profiles (VAP) methodology (VAP Diagnostics Lab, Inc., Birmingham, AL), a form of rapid ultracentrifugation, was used to directly measure the cholesterol concentration in LDL (LDL-C_D). In brief, the VAP uses single vertical spin, density gradient ultracentrifugation to directly measure total cholesterol, HDL-C, LDL-C, VLDL-C, and other lipoprotein cholesterol parameters in under one hour. The VAP methodology has been described in full previously.[32] Triglyceride levels were directly measured using the Abbott ARCHITECT C-8000 system (Abbott Laboratories, Abbott Park, IL). Accuracy of VAP was reviewed yearly using random split-sample comparisons with β-quantification at the Washington University in St. Louis Core Laboratory for Clinical Studies. Directly measured TG concentrations were compared to samples from the University of Alabama School of Medicine for quality assessment.

Friedewald estimated LDL-C (LDL-C_F) was calculated as TC - HDL-C - TG/5 in mg/dL. The novel estimation of LDL-C (LDL-C_N) was calculated as TC - HDL-C - TG/adjustable factor. The adjustable factors were derived from our previously reported method, whereby TG and non-HDL-C were used to assign one of 180 different patient-specific factors to estimate VLDL-C (Supplemental Table 1).[20]

Statistical analysis

All analyses were conducted separately in the fasting and non-fasting groups. Median LDL-C_D values were compared between the two groups stratified by clinical guideline cutpoints for LDL-C: <70, 70-99, 100-129, 130-159, 160-189, and ≥190 mg/dL.[15,28-30] Distributions in the TG:VLDL-C ratio based on fasting status were examined. To assess population-level correlation

between estimated and directly measured LDL-C based on fasting status, we determined via linear regression (R^2) the extent of variation in estimated LDL-C explained by LDL-C_D.

Patient-level accuracy between estimated LDL-C (LDL-C_F or LDL-C_N) and LDL-C_D was compared across the clinical LDL-C cutpoints and classified by fasting status. Accuracy was expressed as the proportion of directly-measured LDL-C falling in the appropriate category of estimated LDL-C (LDL-C_F or LDL-C_N), as this reflects how clinicians make decisions. For LDL-C <70 mg/dL, we decided *a priori* to examine accuracy based on the following TG levels used in our prior studies: <100, 100-149, 150-199, and 200-399 mg/dL.[20] Accuracy between the LDL-C estimation methods was compared using a two-sample test of proportions. We further evaluated the absolute magnitude of error with each method by assessing the percentage of patients whose estimated LDL-C was <5, 5-9, 10-19, 20-29, and ≥30% or <5, 5-9, 10-19, 20-29, and ≥30 mg/dL of LDL-C_D via ultracentrifugation. Poisson regressions were used to assess the interaction between fasting status and LDL-C method, stratified by magnitude of error. All p-values reported are two-sided.

Statistical analysis was performed with Stata (StataCorp), version 11.0.

Results

Population characteristics

Fasting (n=959,153; 62%) and non-fasting (n=586,481; 38%) participants were similar with respect to selected demographics (Table 1). Both groups had a median age around 55 years and were predominantly women. Directly measured median lipid values were almost identical between the two groups, with the exception of a 15 mg/dL higher median TG level in non-fasting patients. There were no differences in the median LDL-C_D values between fasting and non-

fasting patients across the clinical LDL-C categories. Furthermore, when stratifying based on TG levels among participants with LDL-C_D <70 mg/dL, median LDL-C_D values were almost identical between the two groups.

Distribution in the TG:VLDL ratio

The median TG:VLDL-C ratio was 4.9 in the fasting group (interquartile range [IQR] 4.3-5.7) and 5.3 in the non-fasting group (IQR 4.6-6.2). The 5th to 95th percentile for fasting TG:VLDL-C ratio was 3.6 to 7.2 compared to 3.7 to 8.0 for non-fasting TG:VLDL-C.

Correlation in estimated vs. measured LDL-C

When including all patients, there was excellent correlation between estimated and directly measured LDL-C using both methods, with minimal differences in R^2 values between the fasting and non-fasting samples (Friedewald R^2: 0.98 fasting vs 0.96 non-fasting; novel R^2: 0.99 fasting vs 0.98 non-fasting). However, Friedewald correlation in participants with LDL-C_D <70 mg/dL was reduced, especially in non-fasting patients (R^2: 0.66 for fasting samples vs 0.60 for non-fasting samples), compared with the novel method (R^2: 0.80 for both fasting and non-fasting samples).

Magnitude of patient-level error

Overall, the percent differences between estimated LDL-C and LDL-C_D were smaller and, across clinical categories, less affected by fasting status when using the novel method compared to Friedewald estimation ($p<0.001$) (Table 2). In particular for estimated LDL-C<70 mg/dL, 32% of fasting patients and 44% of non-fasting patients had 10% or greater differences between estimated LDL-C and LDL-C_D with the Friedewald equation. This is in comparison to 9% and 10% of patients, respectively, who had ≥10% differences between LDL-C_N and LDL-C_D.

With respect to magnitude of error in mg/dL, a similar overall pattern was observed (Tables 3, 4). In those with LDL-C <70 mg/dL, 19% of fasting and 30% of non-fasting patients had ≥10 mg/dL differences between Friedewald LDL-C and LDL-C_D (Table 3). Only 2-3% of patients had similar degrees of error with the novel method regardless of fasting status. At TG levels 200-399 mg/dL (Table 4), 73% of fasting and 81% of non-fasting patients had ≥10 mg/dL differences between LDL-C_F and LDL-C_D, compared to 25% and 20% of patients respectively with LDL-C_N.

Accuracy in clinical categorization

With both fasting and non-fasting samples, accuracy was higher using the novel method across all LDL-C categories (range: 87-94%) compared to the Friedewald equation (range: 71-93%) ($p \leq 0.001$) (Figure 1a). Accuracy decreased as LDL-C decreased for both methods. However, accuracy in LDL-C_N was less affected by fasting status, with only 2% or smaller differences in accuracy between the fasting and non-fasting groups across the clinical LDL-C groups. With estimated LDL-C <70 mg/dL, the difference in accuracy between the LDL-C_N in fasting (94%) and non-fasting (92%) groups was smaller than for LDL-C_F (78% and 71%, respectively). The percentage of patients moving into higher or lower estimated LDL-C groups is provided as Supplemental Table 2.

Within the estimated LDL-C <70 mg/dL group, accuracy further decreased as TG levels increased for both fasting and non-fasting samples (Figure 1b), particularly for Friedewald estimation (range: 37-96%) compared to the novel method (range: 82-94%). Across the TG categories, non-fasting samples had lower rates of accuracy compared to fasting samples for both methods. Yet, even with TG 200-399 mg/dL in non-fasting patients, LDL-C_N <70 mg/dL accuracy was superior to LDL-C_F (82% vs. 37%) ($p<0.001$).

Discussion

In the first analysis of its kind using a validation sample from the Very Large Database of Lipids study over 3,000 times larger than Friedewald et al's original derivation dataset, we assessed the magnitude of error in LDL-C estimation and accuracy in clinical classification based on fasting status. To our knowledge, no prior study has evaluated the impact of fasting status on novel LDL-C estimation. We found that Friedewald estimation of LDL-C in non-fasting samples leads to greater errors of ≥10% or ≥10 mg/dL and greater misclassification based on standard LDL-C clinical cutpoints compared to Friedewald estimation in fasting samples, particularly at low LDL-C and high TG. In contrast to Friedewald estimation, fasting status had a relatively minimal effect on LDL-C classification with the novel method, which minimizes error and maintains substantially greater accuracy in clinical classification across the range of LDL-C and TG values.

Comparison to literature

LDL-C has been of long-standing clinical importance in cardiovascular risk assessment and treatment decision-making as reflected in worldwide guidelines. Yet, international guidelines remain divided with recommending LDL-C estimation in the fasting state. While the recent 2016 joint consensus statement from the European Atherosclerosis Society and the European Federation of Clinical Chemistry and Laboratory Medicine (EAS-EFCLM) favors routine non-fasting lipid evaluation, the 2013 American College of Cardiology/American Heart Association Prevention Guideline suggests otherwise and instead prefers fasting lipid panels.[1,15]

Guidelines and expert recommendations will continue to evolve.[1–6,33] However, there is strong interest in non-fasting lipid assessment and many patients are having non-fasting lipid profiles. In our data, 38% of patients across the United States had their lipid assessments

performed in the non-fasting state. This proportion may increase in response to recent expert recommendations and may already be higher in other regions of the world.[1]

A key question worth further scrutiny is the impact of non-fasting on LDL-C accuracy and clinical decision-making. To lend support for non-fasting Friedewald estimation, the EAS-EFCLM statement cited multiple population-based studies which found 8-19% increases in TG levels in non-fasting samples with resultant decreases of 4-25% in Friedewald-estimated LDL-C.[7,12,34-36] However, averaging the differences in LDL-C between fasting and non-fasting patients across a population masks trends within particular patient groups. In our study, error in Friedewald estimation within non-fasting samples appeared to increase compared to fasting samples as LDL-C decreased, yet these differences were relatively preserved with the novel method.

Furthermore, data from the Copenhagen City Heat Study constituted a core argument in support of non-fasting lipid assessment in the joint EAS-EFCLM statement.[1,34] Because there was a minimal difference in R^2 values between directly measured LDL-C and Friedewald estimated LDL-C in fasting ($R^2 = 0.87$) and non-fasting ($R^2 = 0.84$) patients, the joint committee argued for the use of routine non-fasting samples.[1]

Yet, this was again a population-based assessment and the problem mainly occurs in the specific setting of low LDL-C and high TG. To this point, we have shown when isolating patients at a lower LDL-C cutpoint (<70 mg/dL), the correlation between estimated and directly measured LDL-C markedly decreases and is affected by fasting status with Friedewald estimation, yet remains high with the novel method regardless of fasting status. Although many people in a study like ours may have high LDL-C levels at a given point in time, where differences in LDL-C accuracy between the methods may have little consequence, many of the

same patients will eventually be treated to lower LDL-C levels where such differences then become more important.

Implications for clinical care

We submit that the central question regarding Friedewald accuracy in non-fasting patients depends on whether non-fasting LDL-C_F misclassifies a significant proportion of patients based on clinical LDL-C cutpoints, as this reflects the way that clinicians and patients make data-driven and guideline-recommended decisions. The EAS-EFCLM statement suggests that minor variations in non-fasting lipid values may only reclassify a few individuals.[1] However, given the approximately 200 million Americans who undergo lipid testing each year, and with treatment guidelines using LDL-C values to initiate and titrate therapies, which LDL-C category an estimated value falls within may have important treatment implications for large numbers of individuals.[37]

We have specifically shown that individual, patient-level misclassification increases substantially with Friedewald estimation using non-fasting samples as LDL-C decreases below 70 mg/dL. In this LDL-C range, there is an overall 7% absolute difference in misclassification rates with non-fasting samples compared to fasting samples. This translates to a misclassification of nearly 1 out of every 14 additional patients if a clinician uses the Friedewald method in the non-fasting state, which may erroneously exclude such patients for initiation or intensification of lipid-lowering therapy. This inaccuracy worsens in the setting of hypertriglyceridemia and is in contrast to the novel method, which appears to largely preserve estimated LDL-C accuracy with non-fasting samples. In the range where the Friedewald equation is most problematic and accuracy is of utmost importance for high-risk patients with secondary prevention goals of LDL-

C <70 mg/dL, only 1 out of every 50 additional patients may be misclassified into a higher LDL-C group when applying the novel equation to non-fasting samples.

With respect to absolute error, nearly ten times as many non-fasting patients with Friedewald-estimated LDL-C <70 mg/dL had errors ≥10 mg/dL in LDL-C calculation when compared to the novel method. This amounts to 1 out of every 3 patients when applying the Friedewald equation. Moreover, with hypertriglyceridemia 200-399 mg/dL at this LDL-C level, nearly four times as many non-fasting patients had errors ≥10 mg/dL with Friedewald-derived LDL-C. With increased variance in the TG:VLDL-C ratio in the post-prandial state, the novel method therefore better accounts for the range of possible TG levels by adjusting the ratio of TG:VLDL-C.

Even within fasting samples, the novel method lends a greater degree of accuracy. In Friedewald et al's original 1972 study consisting of 448 patients, lipid samples were analyzed in the fasting state to reduce fluctuating TG levels.[16] However, even after controlling for any post-prandial TG variation, there still exists large variance in the TG:VLDL-C ratio. This is an unavoidable byproduct of lipid metabolism. Other factors such as diabetes and insulin resistance, presence of obesity and metabolic syndrome, and genetic predisposition account for inherent causes of TG variation.[38] Assuming a fixed relationship of 5 across a population between TG and VLDL-C does not account for these issues in individuals, even in the fasting state, which explains the greater precision of the adaptable approach.

We acknowledge that lipoprotein(a) (Lp(a)) values may be elevated and therefore affect the accuracy of LDL-C given that the conventional Friedewald and Centers for Disease Control and Prevention (CDC) definition of LDL-C incorporates the cholesterol contents of Lp(a) as well as intermediate-density lipoprotein. Although Lp(a)-cholesterol is a relatively small fraction of

LDL-C in most individuals, it will consist of a higher fraction in those with high Lp(a). The novel method of LDL-C estimation is calculated using the standard Friedewald and CDC definition of LDL-C, and the estimated portion of VLDL-C is not dependent on Lp(a). Furthermore, Lp(a) appears to be unaffected by fasting status, and therefore should be relatively constant between the fasting and non-fasting states.[40]

Limitations

The relative strengths of our study include its sample size and the generalizability of our results. We have previously shown that the population distributions of TC, HDL-C, TG, Friedewald LDL-C, and non-HDL-C from the Very Large Database of Lipids cohort are almost identical to those from the US population-representative National Health and Nutritional Examination Survey (NHANES).[20,32]

Limitations include not knowing the exact time of fasting for each patient despite protocol calling for 10-12 hours of fasting prior to sample collection. However, previous studies have suggested that the timing of fasting has minimal effect on the initial absolute increase in TG concentration and subsequent LDL-C estimation in the post-prandial period.[10,35,38-39] The VLDL database furthermore does not report the medications used by each patient at the time of lipid collection. Nevertheless, clinical guidelines continue to support the use of longitudinal LDL-C assessment either in the fasting or non-fasting state regardless of any concomitant lipid-lowering therapy. While we have age and sex available for patients - two factors which may affect TG levels and subsequently the variance in TG:VLDL-C ratios - other factors such as insulin resistance, race, and obesity were not available for analysis. We further used the first available lipid sample for patients. Intra-individual variation between sequential samples may affect LDL-

C classification. However, our sample size ensured adequate numbers of patients for analysis in each arm of the study.

Conclusions

In a large cross-sectional population, we have shown for the first time that estimated LDL-C accuracy and error are largely preserved with novel adaptable LDL-C estimation regardless of fasting status. This is in contrast to the fixed Friedewald method, where fasting status may have important clinical implications on estimated LDL-C accuracy. With the recent trend towards non-fasting lipid assessment, and with the continued focus on LDL-C in global clinical guidelines, the novel method can provide more precise non-fasting LDL-C estimation particularly for patients with lower LDL-C and higher TG levels. In addition to stimulating further study, these findings may have immediate relevance to guideline committees, laboratory leadership, clinicians and patients seeking to make decisions based on the most precise information possible.

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Disclosures

Drs. Martin and Jones are listed as co-inventors on a pending patent filed by Johns Hopkins University for the novel method of LDL cholesterol estimation. Dr. Jones has served as an

advisor to Sanofi/Regeneron. Dr. Martin has served as a consultant to Quest Diagnostics, Sanofi/Regeneron, Amgen, and the Pew Research Center. The other authors report no conflicts.

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Table 1. Study population characteristics and lipid values, stratified by fasting status

	Fasting (n=959,153)	Non-fasting (n=586,481)
Characteristic		
Age, y, Median (IQR)	56 (46-67)	55 (43-66)
Male, No. (%)	445,457 (47)	247,013 (42)
Lipid value, mg/dL, Median (IQR)		
Total cholesterol	195 (165-226)	195 (166-226)
HDL-C	51 (42-63)	51 (42-63)
TG	110 (73-143)	125 (87-182)
Measured LDL-C	116 (91-143)	115 (91-142)
Friedewald LDL-C	115 (90-142)	112 (87-139)
Novel LDL-C	117 (92-143)	115 (91-142)
Number of patients by LDL-C_D categories, No. (%)		
<70	78,458 (8)	49,283 (8)
70-99	228,264 (24)	140,881 (24)
100-129	296,041 (31)	182,541 (31)
130-159	215,476 (23)	129,577 (22)
160-189	97,725 (10)	58,202 (10)
≥190	43,124 (5)	25,938 (4)
LDL-C_D levels by LDL-C categories, mg/dL, Median (IQR)		
<70	60 (53-65)	60 (52-65)
70-99	86 (79-93)	86 (79-93)
100-129	113 (106-121)	113 (106-121)
130-159	141 (135-149)	141 (135-149)
160-189	170 (164-178)	170 (164-178)
≥190	205 (195-221)	205 (195-221)
Number of patients with LDL-C_D<70 by TG levels, No. (%)		
<100	42,972 (55)	23,804 (48)
100-149	20,179 (26)	13,024 (26)
150-199	8,173 (10)	6,257 (13)
200-399	7,134 (9)	6,198 (13)
TG levels, mg/dL, for patients with LDL-C_D<70, Median (IQR)		
<100	69 (55-83)	70 (56-84)
100-149	119 (109-132)	120 (109-133)
150-199	169 (159-182)	171 (159-183)
200-399	250 (221-297)	251 (221-299)

IQR: interquartile range; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; LDL-C_D: directly-measured LDL; TG: triglyceride; mg/dL: milligrams per deciliter; y: years; No: number

Table 2. Percentage of patients by percent error between estimated LDL-C (LDL-CF or LDL-CN) and LDL-CD

Estimated LDL-C*†	<5% error		5-9% error		10-19% error		20-29% error		≥30% error	
	Fasting	Non-fasting	Fasting	Non-fasting	Fasting	Non-fasting	Fasting	Non-fasting	Fasting	Non-fasting
LDL-C_F: <70	43.5 (43.2-43.8)	34.5 (34.1-34.8)	24.1 (23.9-24.4)	21.4 (21.1-21.8)	19.4 (19.1-19.6)	22.1 (21.8-22.4)	7.9 (7.7-8.0)	11.9 (11.7-12.2)	5.1 (5.0-5.2)	10.0 (9.8-10.2)
LDL:C_N: <70	76.5 (76.1-76.8)	72.1 (71.7-72.5)	15.0 (14.7-15.2)	17.9 (17.6-18.2)	6.6 (6.5-6.8)	8.0 (7.8-8.2)	1.3 (1.2-1.3)	1.3 (1.2-1.4)	0.7 (0.6-0.7)	0.7 (0.7-0.8)
LDL-C_F: 70-99	66.5 (66.4-66.7)	57.4 (57.1-57.6)	21.8 (21.6-22.0)	22.4 (22.2-22.7)	9.9 (9.8-10.0)	15.5 (15.3-15.7)	1.6 (1.5-1.6)	4.2 (4.1-4.3)	0.2 (0.2-0.2)	0.5 (0.5-0.5)
LDL-C_N: 70-99	85.2 (85.0-85.3)	82.5 (82.3-82.7)	10.7 (10.6-10.8)	13.1 (12.9-13.2)	3.2 (3.1-3.3)	3.5 (3.4-3.6)	3.2 (3.1-3.3)	3.5 (3.4-3.6)	0.4 (0.4-0.4)	0.4 (0.3-0.4)
LDL-C_F: 100-129	80.7 (80.5-80.8)	71.4 (71.2-71.6)	14.6 (14.5-14.8)	18.3 (18.1-18.4)	4.3 (4.2-4.4)	9.2 (9.1-9.4)	0.3 (0.3-0.3)	1.0 (0.9-1.0)	0.1 (0.1-0.1)	0.1 (0.1-0.1)
LDL-C_N: 100-129	88.9 (88.8-89.0)	86.7 (86.6-86.9)	8.3 (8.2-8.4)	10.5 (10.4-10.6)	2.2 (2.1-2.2)	2.2 (2.2-2.3)	0.4 (0.4-0.4)	0.3 (0.3-0.4)	0.3 (0.3-0.3)	0.3 (0.2-0.3)
LDL-C_F: 130-159	87.9 (87.8-88.0)	80.3 (80.1-80.6)	10.0 (9.9-10.2)	14.5 (14.3-14.7)	1.9 (1.8-1.9)	4.9 (4.8-5.0)	0.1 (0.1-0.1)	0.2 (0.1-0.2)	0.1 (0.1-0.1)	0.1 (0.1-0.1)
LDL-C_N: 130-159	90.6 (90.5-90.7)	88.9 (88.7-89.1)	7.0 (6.9-7.1)	8.8 (8.7-9.0)	1.9 (1.8-1.9)	1.8 (1.7-1.9)	0.3 (0.3-0.3)	0.2 (0.2-0.3)	0.2 (0.2-0.2)	0.2 (0.2-0.2)
LDL-C_F: 160-189	90.9 (90.7-91.1)	85.2 (84.9-85.5)	7.7 (7.5-7.9)	12.0 (11.7-12.3)	1.2 (1.1-1.2)	2.6 (2.4-2.7)	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.2 (0.1-0.2)
LDL-C_N: 160-189	91.0 (90.9-91.2)	89.1 (88.9-89.4)	6.8 (6.6-6.9)	8.6 (8.4-8.9)	1.7 (1.7-1.8)	1.8 (1.7-1.9)	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.2 (0.2-0.2)	0.2 (0.2-0.3)
LDL-C_F: ≥190	91.1 (90.8-91.4)	88.1 (87.7-88.5)	7.1 (6.9-7.3)	9.7 (9.4-10.1)	1.2 (1.1-1.3)	1.5 (1.4-1.7)	0.2 (0.2-0.2)	0.2 (0.1-0.2)	0.4 (0.3-0.4)	0.4 (0.4-0.5)
LDL-C_N: ≥190	90.1 (89.8-90.3)	89.4 (89.0-89.8)	7.1 (6.8-7.3)	7.7 (7.4-8.0)	2.1 (1.9-2.2)	2.0 (1.8-2.2)	0.3 (0.3-0.4)	0.3 (0.2-0.4)	0.5 (0.4-0.6)	0.6 (0.5-0.7)

*Estimated LDL-C values in mg/dL; LDL-C_F: Friedewald LDL-C; LDL-C_N: Novel LDL-C; †Overall, the novel method resulted in more patients in the <10% error group and, across clinical categories, was less affected by fasting status (p<0.001)

Table 3. Percentage of patients by absolute magnitude of error between estimated LDL-C (LDL-CF or LDL-CN) and LDL-CD

Estimated LDL-C*†	<5 mg/dL error		5-9 mg/dL error		10-19 mg/dL error		20-29 mg/dL error		>30 mg/dL error	
	Fasting	Non-fasting	Fasting	Non-fasting	Fasting	Non-fasting	Fasting	Non-fasting	Fasting	Non-fasting
LDL-C_F: <70	61.5 (61.2-61.9)	50.2 (49.8-50.6)	19.3 (19.1-19.6)	19.7 (19.4-20.0)	13.6 (13.3-13.8)	18.4 (18.1-18.7)	4.3 (4.2-4.4)	8.1 (7.9-8.3)	1.3 (1.2-1.3)	3.6 (3.5-3.8)
LDL:C_N: <70	90.1 (89.8-90.3)	88.0 (87.7-88.3)	7.7 (7.5-7.8)	9.3 (9.1-9.6)	2.1 (2.0-2.2)	2.5 (2.3-2.6)	0.1 (0.1-0.2)	0.2 (0.1-0.2)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
LDL-C_F: 70-99	72.0 (71.8-72.2)	61.9 (61.7-62.2)	17.8 (17.6-17.9)	19.4 (19.2-19.6)	8.4 (8.3-8.5)	13.4 (13.2-13.6)	1.6 (1.6-1.7)	4.2 (4.1-4.3)	0.2 (0.2-0.3)	1.1 (1.1-1.2)
LDL-C_N: 70-99	88.9 (88.8-89.0)	86.4 (86.2-86.6)	8.5 (8.3-8.6)	10.7 (10.5-10.8)	2.2 (2.2-2.3)	2.6 (2.5-2.7)	0.3 (0.3-0.3)	0.3 (0.3-0.3)	0.1 (0.1-0.1)	0.1 (0.1-0.1)
LDL-C_F: 100-129	75.4 (75.2-75.5)	66.0 (65.8-66.2)	17.3 (17.2-17.5)	19.3 (19.1-19.5)	6.2 (6.1-6.3)	11.2 (11.0-11.3)	1.0 (0.9-1.0)	2.9 (2.8-2.9)	0.2 (0.1-0.2)	0.6 (0.6-0.7)
LDL-C_N: 100-129	86.5 (86.3-86.6)	83.1 (83.0-83.3)	10.3 (10.2-10.4)	13.0 (12.9-13.2)	2.7 (2.6-2.7)	3.3 (3.2-3.4)	0.4 (0.4-0.4)	0.4 (0.3-0.4)	0.2 (0.2-0.2)	0.2 (0.2-0.2)
LDL-C_F: 130-159	75.4 (75.2-75.6)	67.2 (66.9-67.5)	18.5 (18.4-18.7)	20.3 (20.1-20.6)	5.3 (5.2-5.4)	9.9 (9.8-10.1)	0.7 (0.6-0.7)	2.1 (2.0-2.2)	0.2 (0.1-0.2)	0.4 (0.4-0.4)
LDL-C_N: 130-159	82.9 (82.8-83.1)	78.9 (78.6-79.1)	12.6 (12.4-12.7)	15.8 (15.6-16.0)	3.7 (3.6-3.7)	4.6 (4.5-4.7)	0.5 (0.5-0.6)	0.5 (0.5-0.6)	0.3 (0.2-0.3)	0.2 (0.2-0.3)
LDL-C_F: 160-189	72.5 (72.2-72.8)	66.3 (65.9-66.7)	21.0 (20.7-21.2)	21.9 (21.5-22.2)	5.7 (5.5-5.8)	9.6 (9.4-9.9)	0.6 (0.6-0.7)	1.7 (1.6-1.8)	0.2 (0.2-0.2)	0.4 (0.4-0.5)
LDL-C_N: 160-189	78.2 (78.0-78.5)	72.9 (72.6-73.3)	15.5 (15.3-15.7)	19.2 (18.8-19.5)	5.1 (4.9-5.2)	6.6 (6.4-6.8)	0.8 (0.7-0.9)	0.9 (0.8-1.0)	0.4 (0.4-0.4)	0.4 (0.4-0.5)
LDL-C_F: ≥190	64.2 (63.8-64.7)	61.5 (60.8-62.1)	25.4 (25.0-25.9)	24.7 (24.1-25.2)	8.3 (8.1-8.6)	11.1 (10.7-11.5)	1.2 (1.1-1.3)	1.9 (1.7-2.0)	0.8 (0.7-0.9)	1.0 (0.9-1.1)
LDL-C_N: ≥190	70.3 (69.8-70.7)	66.2 (65.6-66.7)	18.8 (18.5-19.2)	21.9 (21.3-22.4)	7.9 (7.7-8.2)	8.9 (8.6-9.3)	1.7 (1.6-1.9)	1.7 (1.5-1.8)	1.3 (1.2-1.4)	1.4 (1.3-1.6)

*Estimated LDL-C values in mg/dL; LDL-C_F: Friedewald LDL-C; LDL-C_N: Novel LDL-C; †Overall, the novel method resulted in more patients in the <10 mg/dL error group and, across clinical categories, was less affected by fasting status ($p<0.001$)

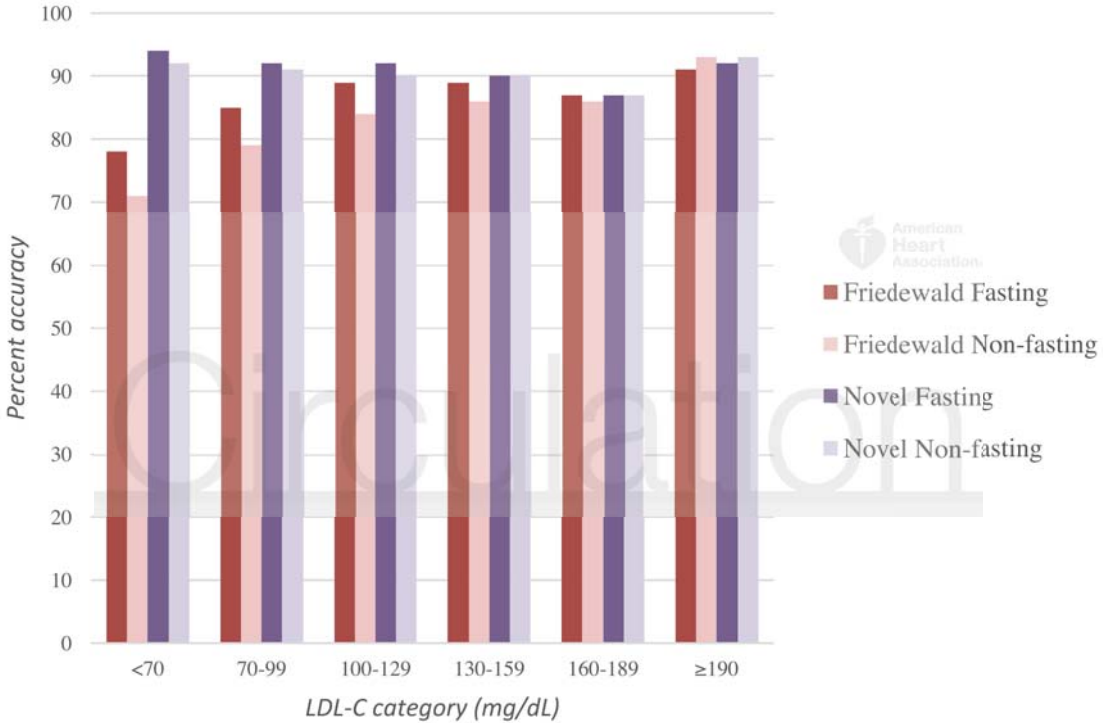
Table 4. Percentage of patients by absolute magnitude of error between estimated LDL-C (LDL-C_F or LDL-C_N) and LDL-C_D

TG categories*†	<5 mg/dL error		5-9 mg/dL error		10-19 mg/dL error		20-29 mg/dL error		>30 mg/dL error	
	Fasting	Non-fasting	Fasting	Non-fasting	Fasting	Non-fasting	Fasting	Non-fasting	Fasting	Non-fasting
TG<100, LDL-C_F<70	91.6 (91.4-91.9)	91.3 (91.0-91.7)	7.7 (7.5-8.0)	8.0 (7.7-8.4)	0.6 (0.5-0.7)	0.6 (0.5-0.7)	0.0 (0.0-0.1)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
TG<100, LDL-C_N<70	95.8 (95.6-96.0)	95.6 (95.3-95.9)	3.4 (3.2-3.5)	3.5 (3.2-3.7)	0.8 (0.8-0.9)	0.9 (0.8-1.1)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
TG 100-149, LDL-C_F<70	71.3 (70.6-71.9)	65.6 (64.7-66.4)	25.3 (24.7-25.9)	30.2 (29.4-31.0)	3.2 (2.9-3.4)	4.0 (3.7-4.4)	0.2 (0.1-0.2)	0.2 (0.1-0.3)	0.1 (0.0-0.1)	0.1 (0.0-0.1)
TG 100-149, LDL-C_N<70	86.9 (86.5-87.4)	87.6 (87.0-88.1)	10.0 (9.6-10.4)	9.8 (9.3-10.3)	2.6 (2.4-2.9)	2.2 (1.9-2.4)	0.4 (0.3-0.5)	0.3 (0.2-0.4)	0.1 (0.0-0.1)	0.1 (0.1-0.2)
TG 150-199, LDL-C_F<70	26.9 (26.0-27.9)	20.0 (19.0-21.0)	43.4 (42.3-44.5)	42.1 (40.9-43.3)	28.5 (27.5-29.5)	36.9 (35.7-38.1)	0.8 (0.7-1.1)	0.8 (0.6-1.1)	0.3 (0.2-0.5)	0.2 (0.1-0.3)
TG 150-199, LDL-C_N<70	70.5 (69.5-71.5)	74.7 (73.6-75.7)	19.7 (18.8-20.6)	18.1 (17.2-19.1)	7.3 (6.8-7.9)	5.5 (4.9-6.1)	1.7 (1.4-2.0)	1.3 (1.0-1.6)	0.8 (0.6-1.0)	0.5 (0.3-0.7)
TG 200-399, LDL-C_F<70	10.0 (9.3-10.7)	7.4 (6.7-8.1)	17.4 (16.5-18.3)	12.0 (11.2-12.8)	46.3 (45.2-47.5)	45.2 (44.0-46.4)	19.9 (19.0-20.9)	26.3 (25.2-27.4)	6.4 (5.9-7.0)	9.1 (8.4-9.9)
TG 200-399, LDL-C_N<70	49.4 (48.2-50.5)	56.8 (55.6-58.1)	25.2 (24.2-26.3)	23.1 (22.1-24.2)	16.7 (15.9-17.6)	13.6 (12.8-14.5)	5.5 (5.0-6.0)	4.1 (3.6-4.6)	3.2 (2.8-3.7)	2.3 (2.0-2.7)

*TG and estimated LDL-C values in mg/dL; LDL-C_F: Friedewald LDL-C; LDL-C_N: ^Novel LDL-C; †Overall, the novel method resulted in more patients in the <10 mg/dL error group and across clinical categories was less affected by fasting status ($p<0.001$) except for TG<100 mg/dL ($p=0.115$)

Figure Legends

Figure 1. Accuracy of estimated LDL-C, stratified by fasting status. Panel A: Accuracy of estimated LDL-C, stratified by LDL-C category and fasting status. Accuracy is expressed as the proportion of directly-measured LDL-C falling in the appropriate category of estimated LDL-C (Friedewald or novel). Friedewald estimation is highlighted in red and the novel equation is highlighted in purple, with darker colors corresponding to fasting samples. Panel B: Accuracy of estimated LDL-C for patients with LDL-C <70 mg/dL, stratified by TG level and fasting status. Accuracy is expressed as the proportion of directly-measured LDL-C falling in the appropriate category of estimated LDL-C (Friedewald or novel). Friedewald estimation is highlighted in red and the novel equation is highlighted in purple, with darker colors corresponding to fasting samples.


100
90
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Percent accuracy
<70
70-99
100-129
130-159
160-189
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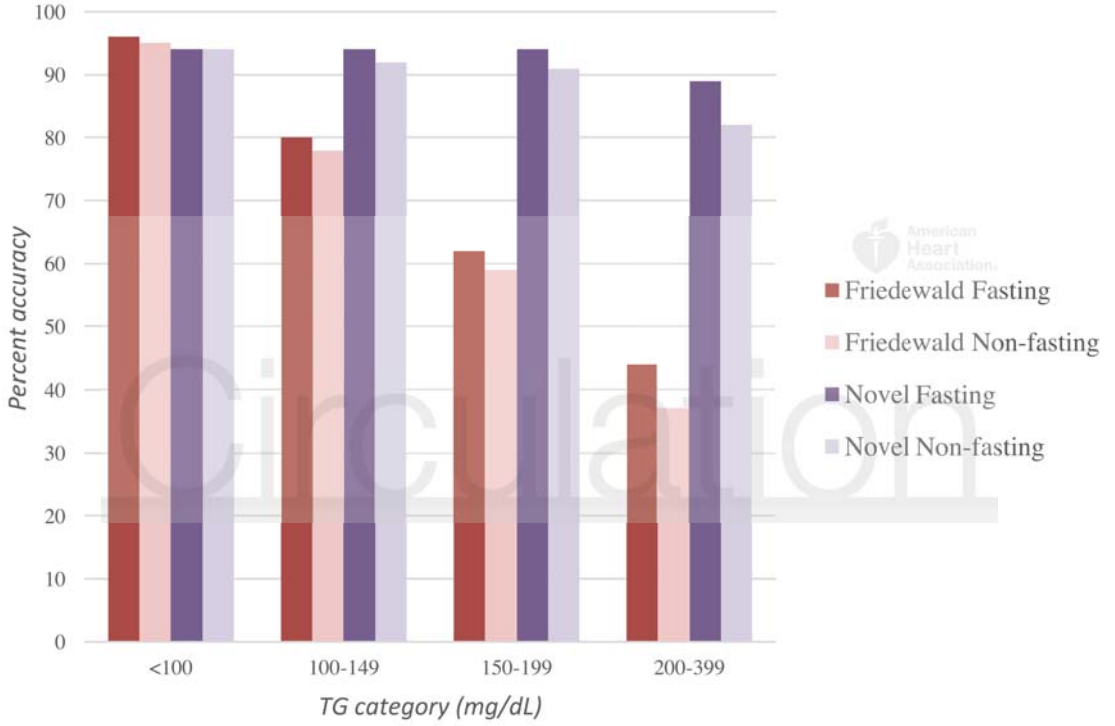
LDL-C category (mg/dL)
Friedewald Fasting
Friedewald Non-fasting
Novel Fasting
Novel Non-fasting



Fasting vs Non-Fasting and Low-Density Lipoprotein-Cholesterol Accuracy

Vasanth Sathiyakumar, Jihwan Park, Asieh Golozar, Mariana Lazo, Renato Quispe, Eliseo Guallar, Roger S. Blumenthal, Steven R. Jones and Seth S. Martin

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SUPPLEMENTAL MATERIAL

Supplemental Table 1: Novel method to derive patient-specific TG:VLDL-C ratios based on TG and non-HDL-C values

Supplemental Table 2: Percentage of patients misclassified into higher or lower LDL-C groups

Supplemental Figure 1: Study patient inclusion and exclusion criteria

Supplemental Table 1. Novel method to derive patient-specific TG:VLDL-C ratios based on TG and non-HDL-C values

	Non-HDL-C, mg/dL					
Triglycerides, mg/dL	<100	100-129	130-159	160-189	190-219	≥220
7-49	3.5	3.4	3.3	3.3	3.2	3.1
50-56	4.0	3.9	3.7	3.6	3.6	3.4
57-61	4.3	4.1	4.0	3.9	3.8	3.6
62-66	4.5	4.3	4.1	4.0	3.9	3.9
67-71	4.7	4.4	4.3	4.2	4.1	3.9
72-75	4.8	4.6	4.4	4.2	4.2	4.1
76-79	4.9	4.6	4.5	4.3	4.3	4.2
80-83	5.0	4.8	4.6	4.4	4.3	4.2
84-87	5.1	4.8	4.6	4.5	4.4	4.3
88-92	5.2	4.9	4.7	4.6	4.4	4.3
93-96	5.3	5.0	4.8	4.7	4.5	4.4
97-100	5.4	5.1	4.8	4.7	4.5	4.3
101-105	5.5	5.2	5.0	4.7	4.6	4.5
106-110	5.6	5.3	5.0	4.8	4.6	4.5
111-115	5.7	5.4	5.1	4.9	4.7	4.5
116-120	5.8	5.5	5.2	5.0	4.8	4.6
121-126	6.0	5.5	5.3	5.0	4.8	4.6
127-132	6.1	5.7	5.3	5.1	4.9	4.7
133-138	6.2	5.8	5.4	5.2	5.0	4.7
139-146	6.3	5.9	5.6	5.3	5.0	4.8
147-154	6.5	6.0	5.7	5.4	5.1	4.8
155-163	6.7	6.2	5.8	5.4	5.2	4.9
164-173	6.8	6.3	5.9	5.5	5.3	5.0
174-185	7.0	6.5	6.0	5.7	5.4	5.1
186-201	7.3	6.7	6.2	5.8	5.5	5.2
202-220	7.6	6.9	6.4	6.0	5.6	5.3
221-247	8.0	7.2	6.6	6.2	5.9	5.4
248-292	8.5	7.6	7.0	6.5	6.1	5.6
293-399	9.5	8.3	7.5	7.0	6.5	5.9
400-13,975	11.9	10.0	8.8	8.1	7.5	6.7

Non-HDL-C: non-high-density lipoprotein-cholesterol; mg/dL: milligrams per deciliter

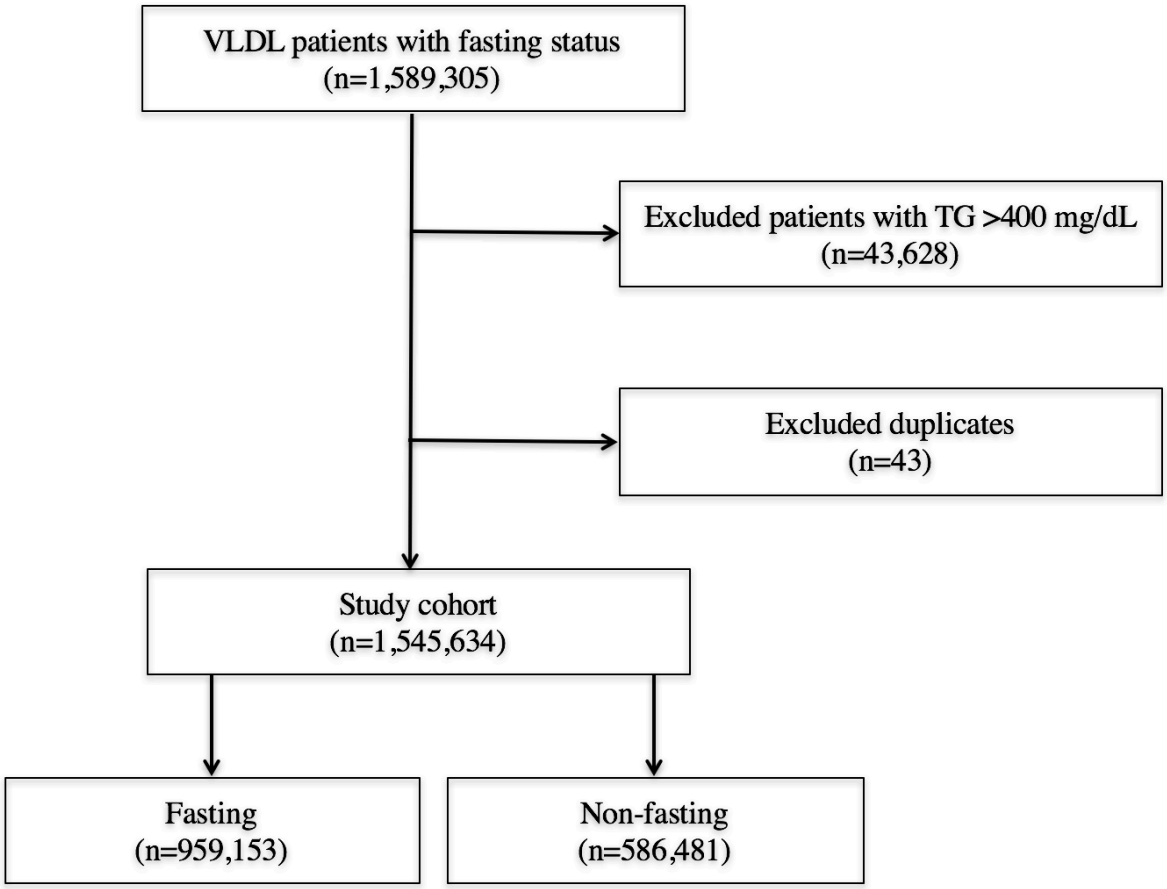
Table adapted with permission from Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, Jones SR. Comparison of a novel method versus the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA*. 2013;310:2061-2068.

Supplemental Table 2. Percentage of patients misclassified into higher or lower LDL-C groups

	Fasting		Non-fasting	
	Downward	Upward	Downward	Upward
Estimated LDL-C*				
$LDL\text{-}C_F$: <70	-	22	-	29
$LDL\text{-}C_N$: <70	-	6	-	8
$LDL\text{-}C_F$: 70-99	2	13	2	19
$LDL\text{-}C_N$: 70-99	3	5	3	7
$LDL\text{-}C_F$:100-129	4	8	3	13
$LDL\text{-}C_N$:100-129	5	4	4	6
$LDL\text{-}C_F$:130-159	6	5	5	10
$LDL\text{-}C_N$:130-159	7	3	6	5
$LDL\text{-}C_F$:160-189	9	4	7	7
$LDL\text{-}C_N$:160-189	10	3	8	5
$LDL\text{-}C_F$: >190	10	-	7	-
$LDL\text{-}C_N$: >190	9	-	7	-

*estimated LDL-C values in mg/dL; $LDL\text{-}C_F$: Friedewald LDL-C; $LDL\text{-}C_N$: Novel LDL-C

Supplemental Figure 1



Legend: Study patient inclusion and exclusion criteria. VLDL: Very Large Database of Lipids. TG: triglycerides; n: number; mg/dL: milligrams per deciliter