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Comparison of Low-Density Lipoprotein Cholesterol Assessment by Martin/Hopkins Estimation, Friedewald Estimation, and Preparative Ultracentrifugation
Insights From the FOURIER Trial

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IMPORTANCE Recent studies have shown that Friedewald underestimates low-density lipoprotein cholesterol (LDL-C) at lower levels, which could result in undertreatment of high-risk patients. A novel method (Martin/Hopkins) using a patient-specific conversion factor provides more accurate LDL-C levels. However, this method has not been tested in proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor–treated patients.

OBJECTIVE To investigate accuracy of 2 different methods for estimating LDL-C levels (Martin/Hopkins and Friedewald) compared with gold standard preparative ultracentrifugation (PUC) in patients with low LDL-C levels in the Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Patients With Elevated Risk (FOURIER) trial.

DESIGN, SETTING, AND PARTICIPANTS The FOURIER trial was a randomized clinical trial of evolocumab vs placebo added to statin therapy in 27 564 patients with stable atherosclerotic cardiovascular disease. The patients' LDL-C levels were assessed at baseline, 4 weeks, 12 weeks, 24 weeks, and every 24 weeks thereafter, and measured directly by PUC when the level was less than 40 mg/dL per the Friedewald method (calculated as non–HDL-C level − triglycerides/5). In the Martin/Hopkins method, patient-specific ratios of triglycerides to very low-density lipoprotein cholesterol (VLDL-C) ratios were determined and used to estimate VLDL-C, which was subtracted from the non–HDL-C level to obtain the LDL-C level.

MAIN OUTCOMES AND MEASURES Low-density lipoprotein cholesterol calculated by the Friedewald and Martin/Hopkins methods, with PUC as the reference method.

RESULTS For this analysis, the mean (SD) age was 62.7 (9.0) years; 2885 of the 12 742 patients were women (22.6%). A total of 56 624 observations from 12 742 patients had Friedewald, Martin/Hopkins, and PUC LDL-C measurements. The median difference from PUC LDL-C levels for Martin/Hopkins LDL-C levels was −2 mg/dL (interquartile range [IQR], −4 to 1 mg/dL) and for Friedewald LDL-C levels was −4 mg/dL (IQR, −8 to −1 mg/dL; $P < .001$). Overall, 22.9% of Martin/Hopkins LDL-C values were more than 5 mg/dL different than PUC values, and 2.6% were more than 10 mg/dL different than PUC levels. These were significantly less than respective proportions with Friedewald estimation (40.1% and 13.3%; $P < .001$), mainly because of underestimation by the Friedewald method. The correlation with PUC LDL-C was significantly higher for Martin/Hopkins vs Friedewald (ρ, 0.918 [95% CI 0.916-0.919] vs ρ, 0.867 [0.865-0.869], $P < .001$).

CONCLUSIONS AND RELEVANCE In patients achieving low LDL-C with PCSK9 inhibition, the Martin/Hopkins method for LDL-C estimation more closely approximates gold standard PUC than Friedewald estimation does. The Martin/Hopkins method may prevent undertreatment because of LDL-C underestimation by the Friedewald method.

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The Friedewald formula to estimate low-density lipoprotein cholesterol (LDL-C) levels was derived from 448 normal or hyperlipidemic individuals more than 4 decades ago, before the existence of current LDL-lowering therapies.[1] The formula's core variable is an estimate of very low-density lipoprotein cholesterol (VLDL-C) as the level of triglycerides (in milligrams per deciliter) divided by 5, while the other 2 components, total and high-density lipoprotein cholesterol (HDL-C), are quantitated. Then, LDL-C is calculated by subtracting VLDL-C and HDL-C from total cholesterol. Accuracy was sufficient for clinical practice and research purposes in a time when estimated VLDL-C was small compared with generally elevated LDL-C levels in the overall population.

However, this is no longer the case because of the advent of effective LDL-C–lowering therapies. As a result of recent clinical trial evidence,[2-4] lower LDL-C targets (eg, <70 mg/dL; to convert to millimoles per liter, multiply by 0.0259) have been incorporated into treatment guidelines. At such levels, the Friedewald formula appears to underestimate LDL-C levels,[5] which could result in the undertreatment of high-risk patients. The gold standard for LDL-C assessment is preparative ultracentrifugation (PUC), a lengthy, highly manual technique requiring significant laboratory skill and expense, which reserves it mainly to research settings. Homogeneous detergent-based automated LDL-C assays, which are sometimes referred to as direct LDL-C assays, are poorly standardized and not optimized for low LDL-C levels.[6,7]

To address the need for more accurate LDL-C estimation at a scale that could be used in routine clinical practice, the Martin/Hopkins algorithm was developed using density gradient ultracentrifugation in a large sample of patients with and without lipid lowering and reflecting a wide range of LDL-C levels, including low levels.[8] It uses the same standard lipid measurements of total and HDL cholesterol and triglycerides as the Friedewald equation does, but it uses a personalized rather than fixed conversion factor in calculating LDL-C levels. Multiple groups in the United States and other countries have validated the Martin/Hopkins LDL-C algorithm.[9-13] However, to our knowledge, no prior published reports have evaluated the Martin/Hopkins algorithm specifically in patients with low LDL-C levels who were treated with an inhibitor of proprotein convertase subtilisin/kexin type 9 (PCSK9). Therefore, we investigated accuracy of the Friedewald and Martin/Hopkins LDL-C estimates compared with PUC in patients with Friedewald LDL-C less than 40 mg/dL in the Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) trial.

Key Points

Question What is the accuracy of low-density lipoprotein cholesterol (LDL-C) by Martin/Hopkins vs Friedewald estimation in patients achieving low LDL-C?

Findings In the FOURIER trial, 22.9% of LDL-C values calculated by the Martin/Hopkins method were more than 5 mg/dL different than the gold standard values and 2.6% varied by more than 10 mg/dL from the gold standard preparative ultracentrifugation values. This was significantly less than the respective proportions with Friedewald estimation (40.1% and 13.3%), which frequently underestimated the LDL-C concentration.

Meaning In the first study of its kind in proprotein convertase subtilisin/kexin type 9 inhibitor–treated patients, our results show that the Martin/Hopkins method may provide a more accurate estimate of LDL-C levels, thereby potentially preventing undertreatment because of LDL-C level underestimation by the Friedewald method.

Methods

In the FOURIER trial, 27 564 patients with stable atherosclerotic cardiovascular disease were randomized to placebo or evolocumab, a monoclonal antibody to PCSK9.[14,15] Eligible patients had LDL-C levels of 70 mg/dL or greater or non-high-density lipoprotein cholesterol (non-HDL-C) levels of 100 mg/dL or greater and were taking high-intensity or moderate-intensity statin therapy. Data collection occurred at 1242 centers in 49 countries from 2013 to 2016. Ethics committee approvals were obtained from relevant local organizations or a central institutional review board within a given country, and each patient provided written informed consent.

Total and HDL cholesterol and triglyceride levels were measured and LDL-C level was calculated at baseline, 4 weeks, 12 weeks, 24 weeks, and every 24 weeks thereafter. Friedewald LDL-C levels were estimated as total cholesterol minus HDL-C minus triglycerides divided by 5 in individuals with triglyceride levels less than 400 mg/dL. The Martin/Hopkins algorithm for LDL-C calculation was applied to the same data to calculate the LDL-C level as total cholesterol minus HDL-C minus triglycerides divided by a personalized factor, which was a patient-specific triglyceride:VLDL-C ratio. This personalized factor, which ranged from 3.1 to 9.5, was selected from a table based on the patient's non-HDL-C and triglyceride values, which were available from the standard lipid profile. Like the Friedewald method, Martin/Hopkins estimations were limited to samples with triglyceride values less than 400 mg/dL. Preparative ultracentrifugation LDL-C ascertainment was performed when the Friedewald LDL-C level was less than 40 mg/dL, using a standardized assay (eMethods in the Supplement). Therefore, the present analyses were restricted to samples with Friedewald LDL-C less than 40 mg/dL.

We assessed differences between estimated LDL-C levels (by the Friedewald and Martin/Hopkins methods) and PUC LDL-C levels, including fifth, 25th, 50th (median), 75th, and 95th percentile differences, and differences were tested using the Wilcoxon matched-pairs signed rank test. Furthermore, we compared proportions with specified differences between estimated LDL-C levels (by the Friedewald and Martin/Hopkins methods) and PUC LDL-C levels by error categories (ie, ≤5.0, 5.1-10.0, 10.1-20.0, 20.1-30.0, and > 30.0 mg/dL). Proportions were compared using the McNemar test for dichotomous variables and marginal homogeneity tests when more than 2 categories existed. Analyses were performed in the overall group and stratified by triglycerides less than 150 vs greater than or equal to 150 mg/dL. We created scatterplots of the 2 LDL-C estimates vs PUC levels, then examined regression lines and correlations. Correlations were compared using Fisher *r*-to-*z* transformations. All analyses were conducted with Stata/

Table 1. Differences Between LDL-C Levels in FOURIER Trial Patients With Friedewald LDL-C Less Than 40 mg/dL, Overall and Stratified by Triglycerides[a]

	LDL-C Level Differences, mg/dL								
	All Patients With Friedewald LDL-C <40 mg/dL[b] (n = 56 624 Observations)			Patients With Friedewald LDL-C <40 mg/dL and TG ≥150 mg/dL (n = 11 991 Observations)			Patients With Friedewald LDL-C <40 mg/dL and TG <150 mg/dL (n = 44 633 Observations)		
Percentile	Martin/Hopkins	Friedewald	*P* Value	Martin/Hopkins	Friedewald	*P* Value	Martin/Hopkins	Friedewald	*P* Value
5th	−7	−15	<.001	−6	−22	<.001	−8	−10	<.001
25th	−4	−8		−1	−14		−5	−6	
50th	−2	−4		2	−10		−2	−3	
75th	1	−1		6	−7		0	−1	
95th	7	3		14	−1		4	3	

Abbreviations: FOURIER, Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Patients With Elevated Risk; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

SI conversion factors: To convert cholesterol to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0113.

[a] Interaction with triglycerides stratified at 150 mg/dL.

[b] Total patients with Friedewald values less than 40 mg/dL numbered 12 742.

Table 2. Proportion of FOURIER Trial Patients With Friedewald LDL-C Less Than 40 mg/dL With Specific Magnitudes of Absolute Errors Between Estimated and Preparative Ultracentrifugation LDL-C Levels, Overall and Stratified by Triglycerides

	Absolute Difference, % (Overestimation, %/Underestimation, %)								
	All Patients With Friedewald LDL-C <40 mg/dL (n = 12 742 Patients; n = 56 624 Observations)			Patients With Friedewald LDL-C <40 mg/dL and TG ≥150 mg/dL (n = 11 991 Observations)			Patients With Friedewald LDL-C <40 mg/dL and TG <150 mg/dL (n = 44 633 Observations)		
Value Range, mg/dL	Martin/Hopkins	Friedewald	*P* Value	Martin/Hopkins	Friedewald	*P* Value	Martin/Hopkins	Friedewald	*P* Value
≤5	77.1 (23.2/53.9)	59.9 (13.0/46.9)	<.001	68.0 (35.0/33.0)	17.2 (2.2/15.1)	<.001	79.5 (20.0/59.5)	71.3 (15.9/55.4)	<.001
>5-10	20.3 (5.2/15.1)	26.8 (0.8/26.1)		21.9 (17.1/4.9)	32.5 (0.4/32.1)		19.9 (2.1/17.8)	25.3 (0.8/24.5)	
>10-20	2.3 (1.9/0.4)	11.7 (0.1/11.7)		8.4 (8.3/0.2)	43.0 (0.3/42.7)		0.6 (0.2/0.5)	3.4 (0.04/3.3)	
>20-30	0.3 (0.3/<0.01)	1.4 (0.02/1.4)		1.3 (1.3/<0.01)	6.6 (0.1/6.5)		0.01 (0.01/<0.01)	0.01 (<0.01/0.01)	
>30	0.1 (0.1/<0.01)	0.1 (<0.01/0.1)		0.3 (0.3/<0.01)	0.7 (<0.01/0.7)		<0.01 (<0.01/<0.01)	<0.01 (<0.01/<0.01)	

Abbreviations: FOURIER, Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Patients With Elevated Risk; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

SI conversion factors: To convert cholesterol to millimoles per liter, multiply by 0.0259; triglycerides to millomoles per liter, multiply by 0.0113.

IC, version 14.2 (StataCorp LP) or SAS, version 9.4 (SAS Institute). Values of $P < .05$ were considered significant. Data analysis occurred from December 2017 to April 2018.

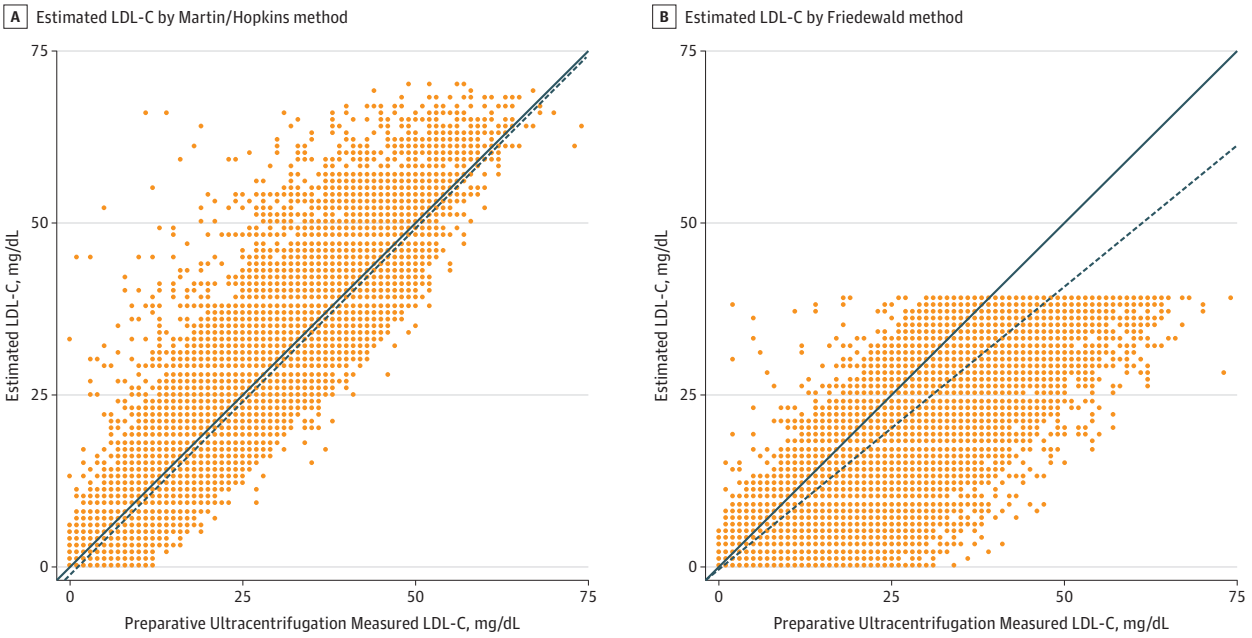
Results

A total of 56 624 observations in 12 742 patients in the FOURIER trial had postbaseline Friedewald LDL-C values less than 40 mg/dL (of which 55 956 measurements [98.8%] were in the evolocumab arm of the trial). A comparison of the patients in this analysis with other patients in the FOURIER trial and the overall trial population is provided in the eTable in the Supplement. For this analysis, the mean (SD) age was 62.7 (9.0) years; 2885 of the 12 742 patients were women (22.6%). The median (interquartile range [IQR]) baseline triglyceride concentrations were 135 (102-185) mg/dL, and 5192 patients (40.7%) had triglyceride levels of 150 mg/dL or more.

Differences between estimated and PUC LDL-C levels are shown in **Table 1.** The median difference for Martin/Hopkins minus PUC LDL-C level was −2 mg/dL (IQR, −4 to 1 mg/dL) and for Friedewald level minus PUC LDL-C level was −4 mg/dL (IQR, −8 to −1 mg/dL; $P < .001$). Differences between the methods were more pronounced in those with triglyceride levels of 150 or greater (Martin/Hopkins: median, 2 mg/dL [IQR, −1 to 6 mg/dL] vs Friedewald: median, −10 mg/dL [IQR, −14 to −7 mg/dL]; $P < .001$).

The proportion of patients with specific levels of errors is shown in **Table 2.** Overall, 12 990 of 56 624 Martin/Hopkins LDL-C values (22.9%) differed by more than 5 mg/dL from PUC values (in either direction) and 1479 of 56 624 (2.6%) differed by more than 10 mg/dL than PUC levels, which were significantly less than respective proportions with Friedewald estimation (22 726 of 56 624 [40.1%] and 7525 of 56 624 [13.3%]; $P < .001$ for each). In patients with triglyceride levels of 150 mg/dL or more, 3835 of 11 991 Martin/Hopkins values (32.0%) differed by more than 5 mg/dL from PUC levels, while 9923 of 11 991 Friedewald values differed by more than 5 mg/dL from PUC levels (82.8%; $P < .001$). In contrast, 9155 of 44 633 Martin/Hopkins values (20.5%) vs 12803 of 44 633 Friedewald values (28.7%) that were more than 5 mg/dL different than PUC levels ($P < .001$) among those with triglyceride values less than 150 mg/dL (interaction between triglyceride level and relative accuracy of LDL-C estimation method, $P < .001$). Moreover, in patients with triglyceride values of 150 mg/dL or greater, 1204 of 11 991 Martin/Hopkins values (10.0%) differed by more than 10 mg/dL from PUC values ($P < .001$) vs 6021 of 11 991 Friedewald values (50.2%; $P < .001$). Among patients with triglyceride values less than 150 mg/dL, 275 of 44 633 Martin/Hopkins values (0.6%) were more than 10 mg/dL different than PUC values, while 1504 of 44 633 had Friedewald values with the same degree of variance (3.4%; $P < .001$; interaction between triglyceride level and relative accuracy of LDL-C estimation method, $P < .001$).

Figure. Scatterplots of Estimated Low-Density Lipoprotein Cholesterol (LDL-C) vs Preparative Ultracentrifugation Measured LDL-C



A line of unity (solid line) and regression line (dashed line) are shown. Note that the study population was defined by Friedewald LDL-C estimations less than 40 mg/dL, whereas Martin/Hopkins and preparative ultracentrifugation values extended to higher levels up to 75 mg/dL. LDL-C indicates low-density lipoprotein cholesterol.

In scatterplots of estimated vs PUC LDL-C levels, Martin/Hopkins LDL-C levels were more evenly distributed around the regression line than Friedewald values were **(Figure)**. The Spearman correlation coefficient with PUC LDL-C levels was significantly higher for Martin/Hopkins vs Friedewald LDL-C levels (Martin/Hopkins: ρ, 0.918 [95% CI, 0.916-0.919] vs Friedewald: ρ, 0.867 [95% CI, 0.865-0.869]; $P < .001$) and Martin/Hopkins LDL-C levels deviated less from observed values (Martin/Hopkins: root mean square [RMS] error, 4.32 [95% CI, 4.25-4.39] vs Friedewald: RMS error, 5.41 [95% CI, 5.34-5.48] mg/dL).

Discussion

To our knowledge, this is the first study comparing the Martin/Hopkins algorithm with the Friedewald equation for estimation of low LDL-C in PCSK9 inhibitor-treated patients. Our results show that, referenced against the gold standard measurement by PUC levels, the Martin/Hopkins method provides a more accurate estimate of LDL-C levels.

National and international guidelines focus on LDL-C, and thus it is used by clinicians in routine clinical practice to guide cholesterol treatment initiation and intensification.[2-4] With new therapeutic options, such as PCSK9 inhibitors, capable of achieving lower LDL-C levels than historically possible,[14,15] the Friedewald equation is prone to underestimation.[5] Although minor underestimation, especially if less than 5 mg/dL, would probably not change the care of the patient, larger magnitudes of underestimation were common. This introduces risk in patient care because of inappropriate withholding, termination, or downtitration of proven LDL-C–reducing and risk-reducing therapy. Prior FOURIER analyses showed a monotonic relationship between achieved LDL-C levels and major cardiovascular outcomes down to LDL-C levels less than 10 mg/dL (by PUC), with no safety concerns over a median of 2.2 years of follow-up time.[15] Our findings may aid in translation of PCSK9 inhibitor trial results to clinical practice by examining a scalable alternative for LDL-C estimation that addresses the problem of underestimation (and hence undertreatment).

Conclusions

In patients achieving low LDL-C levels (<40 mg/dL), with PCSK9 inhibition, the Martin/Hopkins method for LDL-C estimation more closely approximates gold standard PUC levels compared with the Friedewald approach to LDL-C estimation. These data suggest that Martin/Hopkins estimation should be the preferred method to estimate LDL-C levels in such intensively treated patients.

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Author Contributions: Drs Giugliano and Sabatine had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Martin, Giugliano, Wasserman, Sabatine.
Acquisition, analysis, or interpretation of data: All authors.
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REFERENCES

1. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18(6):499-502.

2. Lloyd-Jones DM, Morris PB, Ballantyne CM, et al. 2017 focused update of the 2016 ACC expert consensus decision pathway on the role of non-statin therapies for LDL-cholesterol lowering in the management of atherosclerotic cardiovascular disease risk: a report of the American College of Cardiology Task Force. *J Am Coll Cardiol*. 2017;70(14):1785-1822.

3. Catapano AL, Graham I, De Backer G, et al; ESC Scientific Document Group. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. *Eur Heart J*. 2016;37(39):2999-3058.

4. Orringer CE, Jacobson TA, Saseen JJ, et al. Update on the use of PCSK9 inhibitors in adults: Recommendations from an Expert Panel of the National Lipid Association. *J Clin Lipidol*. 2017;11(4):880-890.

5. Martin SS, Blaha MJ, Elshazly MB, et al. Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. *J Am Coll Cardiol*. 2013;62(8):732-739.

6. Miller WG, Myers GL, Sakurabayashi I, et al. Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. *Clin Chem*. 2010;56(6):977-986.

7. Miller WG, Waymack PP, Anderson FP, Ethridge SF, Jayne EC. Performance of four homogeneous direct methods for LDL-cholesterol. *Clin Chem*. 2002;48(3):489-498.

8. Martin SS, Blaha MJ, Elshazly MB, et al. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA*. 2013;310(19):2061-2068.

9. Meeusen JW, Lueke AJ, Jaffe AS, Saenger AK. Validation of a proposed novel equation for estimating LDL cholesterol. *Clin Chem*. 2014;60(12):1519-1523.

10. Lee J, Jang S, Son H. Validation of the Martin method for estimating low-density lipoprotein cholesterol levels in Korean adults: Findings from the Korea national health and nutrition examination survey, 2009-2011. *PLoS One*. 2016;11(1):e0148147.

11. Chaen H, Kinchiku S, Miyata M, et al. Validity of a novel method for estimation of low-density lipoprotein cholesterol levels in diabetic patients. *J Atheroscler Thromb*. 2016;23(12):1355-1364.

12. Kang M, Kim J, Lee SY, Kim K, Yoon J, Ki H. Martin's equation as the most suitable method for estimation of low-density lipoprotein cholesterol levels in Korean adults. *Korean J Fam Med*. 2017;38(5):263-269.

13. Sathiyakumar V, Park J, Golozar A, et al. Fasting versus nonfasting and low-density lipoprotein cholesterol accuracy. *Circulation*. 2018;137(1):10-19.

14. Sabatine MS, Giugliano RP, Keech AC, et al; FOURIER Steering Committee and Investigators. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376(18):1713-1722.

15. Giugliano RP, Pedersen TR, Park JG, et al; FOURIER Investigators. Clinical efficacy and safety of achieving very low LDL-cholesterol concentrations with the PCSK9 inhibitor evolocumab: a prespecified secondary analysis of the FOURIER trial. *Lancet*. 2017;390(10106):1962-1971.