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Comparison of LDL Cholesterol Concentrations by Friedewald Calculation and Direct Measurement in Relation to Cardiovascular Events in 27 331 Women

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BACKGROUND: National Cholesterol Education Program (NCEP) guidelines recommend development of direct assays for LDL cholesterol (LDL-C) measurement, but it is unclear how these assays compare with Friedewald calculation in predicting cardiovascular disease (CVD).

METHODS: In a study of 27 331 healthy women with triglycerides ≤4.52 mmol/L (≤400 mg/dL), baseline fasting Friedewald LDL-C was compared with fasting and nonfasting direct homogenous measurement for incident CVD during an 11-year period.

RESULTS: Fasting LDL-C measurements obtained by the 2 methods were highly correlated ($r = 0.976$, $P < 0.001$). Compared with fasting Friedewald LDL-C, mean fasting direct LDL-C was 0.15 mmol/L (5.6 mg/dL) lower and nonfasting direct LDL-C 0.30 mmol/L (11.5 mg/dL) lower, both $P < 0.0001$. The adjusted hazard ratio per 1-SD increment was 1.23 [95% CI 1.15–1.32; 1-SD 0.88 mmol/L (34.1 mg/dL)] for fasting direct LDL-C and 1.22 [95% CI 1.14–1.30; 1-SD 0.90 mmol/L (34.9 mg/dL)] for fasting Friedewald. Nonfasting LDL-C was not associated with CVD by either method. Fasting LDL-C measurements fell into the same NCEP risk category with either method for 79.3% of participants, whereas they differed by 1 NCEP category for 20.7% of participants, with most classified into a lower-risk category by direct LDL-C.

CONCLUSIONS: The association of LDL-C with CVD by the 2 methods was nearly identical in fasting samples. However, the lower direct LDL-C concentrations may misclassify many individuals into a lower NCEP category. Moreover, the lack of association of nonfasting direct LDL-C with CVD raises questions regarding the clinical utility of a direct assay for LDL-C in nonfasting blood samples.

According to the third report of the National Cholesterol Education Program (NCEP)[8] Adult Treatment Panel, LDL cholesterol (LDL-C) is the primary target for the diagnosis and treatment of hypercholesterolemia *(1)*. The common approach for determining LDL-C concentration in the clinical laboratory is the Friedewald calculation *(2)*, which derives LDL-C from total cholesterol, HDL cholesterol, and triglycerides in the fasting state. Although this method is routinely used and convenient for clinical practice, it is not recommended for use in nonfasting blood samples or the presence of hypertriglyceridemia [>4.52 mmol/L (400 mg/dL)] or type III hyperlipoproteinemia. For these reasons, an expert panel of the NCEP in 1995 recommended the development of direct methods for the measurement of LDL-C *(3, 4)*. In addition, the Friedewald calculation of LDL-C requires 3 primary measurements (total cholesterol, HDL cholesterol, and triglycerides), potentially decreasing the accuracy and precision of the derived cholesterol concentration. Direct assays are currently used in clinical laboratories, but evaluations of these assays were done in small cross-sectional or retrospective studies, with scarce information comparing the association of LDL-C measured directly with Friedewald calculation in association with clinical events *(5)*.

We conducted this study to evaluate the association of baseline LDL-C concentrations as determined by a direct assay compared with Friedewald calculation in predicting incident cardiovascular disease (CVD) events in a prospective cohort of more than 27 000 ini-

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[8] Nonstandard abbreviations: NCEP, National Cholesterol Education Program; LDL-C, LDL cholesterol; CVD, cardiovascular disease; HR, hazard ratio.

tially healthy women. We also tested whether the direct assay provides useful information when performed on nonfasting blood samples.

Materials and Methods

STUDY POPULATION

Study participants were enrolled in the Women's Health Study, a randomized, double-blinded, placebo-controlled clinical trial of low-dose aspirin and vitamin E in the primary prevention of CVD and cancer in US female healthcare professionals *(6–8)*. Eligible participants were apparently healthy women, age 45 years or older, who were free of self-reported CVD or cancer at study entry (1992–1995), with follow-up for incident CVD through February 2006. At the time of enrollment, participants gave written informed consent and completed questionnaires on demographics, medical history, medications, and lifestyle factors. They were also asked to provide a blood sample. Participants were requested, but not required, to have a fasting sample drawn in the morning before eating and to report the number of hours since their last meal before the blood draw and the time of day of the blood draw. Of the 28 023 women from whom we obtained baseline blood samples, we excluded 86 with missing baseline lipid values, 1 whose calculated Friedewald LDL-C was <0, and another 605 with triglyceride concentrations >4.52 mmol/L (400 mg/dL), resulting in 27 331 women for analysis. Samples from participants whose last meal was 8 h or more before their blood draw comprised the fasting sample (n = 19 777), those from participants who had eaten within 8 h of their blood draw comprised the nonfasting sample (n = 6165); another 1389 samples were from participants whose fasting status was unknown. The study was approved by the institutional review boards of the Brigham and Women's Hospital (Boston, MA).

BASELINE LIPID MEASUREMENTS

EDTA blood samples were obtained at the time of enrollment and stored in vapor-phase liquid nitrogen (−170 °C). Subsequently, in a laboratory (N. Rifai) certified by the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization program, baseline samples were thawed and analyzed for lipid concentrations. Direct measurements of concentrations of total cholesterol, HDL-C, LDL-C, and triglycerides were simultaneously performed on a Hitachi 917 analyzer with reagents and calibrators from Roche Diagnostics. LDL-C was determined by a homogenous direct method from Roche Diagnostics. The between-assay CVs for direct LDL-C concentrations of 2.33, 2.75, and 3.34 mmol/L (90, 106, and 129 mg/dL) were 3.01%, 2.34%, and 2.18%, respectively. Total cholesterol was assayed enzymatically. At total cholesterol concentrations of 3.44 and 7.25 mmol/L (133 and 280 mg/dL), the CVs were 1.7% and 1.6%, respectively. The concentration of HDL-C was determined by use of a direct enzymatic colorimetric assay. HDL-C at the concentrations of 0.70 and 1.42 mmol/L (27 and 55 mg/dL) had CVs of 3.3% and 1.7%, respectively. Triglycerides were measured enzymatically with correction for endogenous glycerol. Triglycerides at concentrations of 0.95 and 2.28 mmol/L (84 and 202 mg/dL) had CVs of 1.8% and 1.7%, respectively. The Friedewald equation was used to calculate LDL-C from total cholesterol, HDL cholesterol, and triglycerides *(2)*. The CV for Friedewald LDL-C was 5.1% over a broad range of LDL-C concentrations.

ASCERTAINMENT OF CVD EVENTS

The primary endpoint of interest was a composite endpoint of incident CVD (nonfatal myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, nonfatal stroke, or cardiovascular death). During the 11-year follow-up period, women reported the endpoints of interest on follow-up questionnaires every 6 or 12 months. All events were adjudicated by an endpoints committee.

STATISTICAL ANALYSIS

Statistical analyses were performed using STATA version 8.2 (STATA Corporation). The distributions of LDL-C concentrations by direct method (fasting and nonfasting) were compared with Friedewald calculation on fasting samples (reference method). *P* values were obtained from Student *t*-tests for means and Kruskal–Wallis tests for medians. Pearson correlation coefficients, scatterplots, and Bland–Altman graphs were used to compare Friedewald and direct LDL-C concentrations. We also used boxplot graphs to examine direct LDL-C concentrations according to time since last meal (2-h intervals).

According to guidelines from the Department of Health and Human Services *(9)*, LDL-C concentrations obtained by Friedewald calculation and direct method were divided into quintiles based on their distributions among women not taking hormone therapy. Cox proportional hazard regression models were used to calculate the hazard ratios (HRs) and 95% CIs for quintiles of fasting and nonfasting direct LDL-C, which were then compared with HRs for quintiles of fasting Friedewald LDL-C. Tests for linear trend were performed using the median value for each quintile.

We also performed regression models with LDL-C coefficients expressed per 1 SD and per 1 mmol/L (38.6 mg/dL). Comparing lipid effects on a 1-SD basis or per quintile relates their effects to their population

Table 1. Distributions of clinical and lipid variables.[a]

Baseline Characteristic	N = 27 331
Age, y	54.7 (7.1)
Current smoking, %	11.5
Hypertension, %	24.7
Diabetes, %	2.5
Postmenopausal status, %	54.3
Postmenopausal hormone use, %	43.5
Fasting status,[b] %	
Fasting	72.4
Nonfasting	22.6
Unknown	5.1
Body mass index, kg/m^2	25.9 (5.0)
Friedewald LDL cholesterol, mmol/L	3.36 (0.90)
Direct LDL cholesterol, mmol/L	3.22 (0.88)
Total cholesterol, mmol/L	5.46 (1.06)
HDL cholesterol, mmol/L	1.40 (0.39)
Triglycerides, mmol/L	1.53 (0.80)

[a] Values are mean (SD) values or percentage.
[b] Because of rounding, percentages may not add up to 100%.

distributions, whereas the 1 mmol/L analysis compares their effects directly, without taking into account differences in their distributions. Finally, to determine the concordance of the 2 methods in classifying risk, we used NCEP categories of risk to classify participants on the basis of their fasting LDL-C concentrations as determined by Friedewald calculation or direct measurement. All *P* values were 2-tailed.

Results

STUDY POPULATION AND DISTRIBUTIONS OF LDL-C

As shown in Table 1, the mean (SD) age of study participants at baseline was 54.7 (7.1) years. Fasting blood samples were available in 19 777 participants (72.4%) and nonfasting samples in 6615 participants (22.6%). The mean lipid concentrations were as expected for a healthy cohort of middle-aged women.

Compared with fasting Friedewald LDL-C (Table 2), mean concentrations of fasting direct LDL-C were lower by 0.15 mmol/L (5.6 mg/dL), and nonfasting direct LDL-C lower by 0.30 mmol/L (11.5 mg/dL), both $P < 0.0001$. Mean nonfasting Friedewald LDL-C [3.24 mmol/L (125 mg/dL), SD 0.88 mmol/L (34.1 mg/dL)] was also lower than fasting Friedewald by 0.17 mmol/L (6.7 mg/dL). In participants with triglycerides ≤2.26 mmol/L (≤200 mg/dL), a similar pattern was observed, with fasting and nonfasting direct LDL-C being lower than fasting Friedewald LDL-C. In participants with triglycerides between 2.26 and 4.52 mmol/L (200 and 400 mg/dL), there was a small

Table 2. Distribution of LDL cholesterol values according to Friedewald calculation and direct assay.

	n	Mean (SD) mmol/L	Median mmol/L	25th, 75th Percentile mmol/L	*P* fasting Friedewald vs fasting direct[a]	*P* fasting Friedewald vs nonfasting direct[a]
All participants						
Friedewald, fasting	19 777	3.40 (0.90)	3.33	2.78, 3.94	<0.0001	
Direct, fasting	19 777	3.26 (0.88)	3.19	2.65, 3.78		
Direct, nonfasting	6165	3.11 (0.86)	3.03	2.51, 3.62		<0.0001
Participants with triglycerides ≤2.26 mmol/L (≤200 mg/dL)						
Friedewald, fasting	16 840	3.36 (0.88)	3.30	2.76, 3.88	<0.0001	
Direct, fasting	16 840	3.20 (0.85)	3.13	2.62, 3.71		
Direct, nonfasting	4899	3.02 (0.81)	2.95	2.45, 3.52		<0.0001
Participants with triglycerides of 2.27–4.52 mmol/L (201–400 mg/dL)						
Friedewald, fasting	2937	3.63 (1.03)	3.54	2.93, 4.24	<0.0001	
Direct, fasting	2937	3.59 (0.98)	3.51	2.93, 4.16		
Direct, nonfasting	1266	3.44 (0.93)	3.36	2.82, 3.96		<0.0001

[a] *P* values were obtained from Student *t*-tests comparing mean concentrations of fasting Friedewald (reference) with either fasting direct (paired *t*-test) or nonfasting direct (unpaired *t*-test).

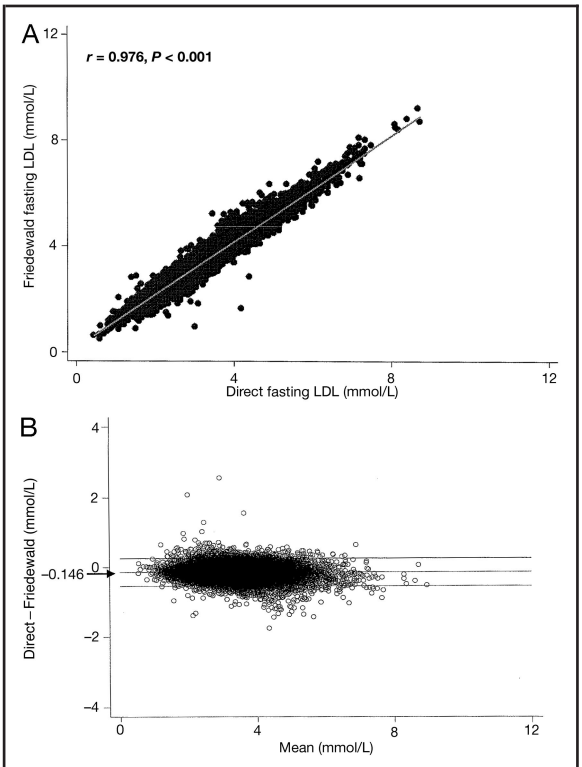

Fig. 1. Scatterplot showing the correlation of Friedewald fasting and direct fasting LDL-C values, Pearson correlation coefficient 0.976, $P < 0.001$ (A) and Bland-Altman plot showing the difference between direct fasting and Friedewald fasting values vs the mean of the 2 (B).

The mean difference (direct minus Friedewald) was −0.146 (95% CI −0.149, −0.143).

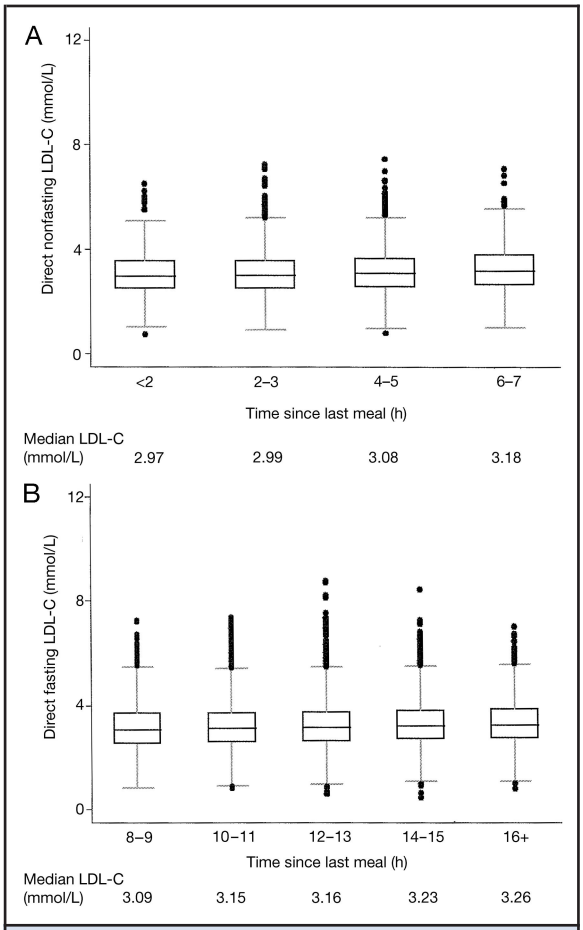

Fig. 2. Boxplots showing the 2.5%, 25%, 50%, 75%, and 97.5% cumulative percentile values and extreme values, according to time since last meal, for direct nonfasting LDL-C (A) and direct fasting LDL-C (B).

difference between fasting concentrations of direct and Friedewald LDL-C [0.04 mmol/L (−1.3 mg/dL), respectively, $P < 0.0001$], whereas nonfasting direct LDL-C was lower by 0.19 mmol/L (7.2 mg/dL) compared with fasting Friedewald ($P < 0.0001$).

Fasting direct LDL-C correlated highly with fasting Friedewald LDL-C in a linear manner (Pearson's correlation coefficient $r = 0.976$, $P < 0.001$) (Fig. 1). Nonfasting direct LDL-C also correlated highly with nonfasting Friedewald LDL-C ($r = 0.972$, $P < 0.001$). The difference between fasting concentrations of direct and Friedewald LDL-C is plotted against their mean in the Bland-Altman graph shown in Fig. 1, right panel. The mean difference in LDL-C between the 2 methods (direct − Friedewald) was −0.146 mmol/L (−5.64 mg/dL) (95% CI −0.149, −0.143 mmol/L [−5.75, −5.53 mg/dL]) in fasting samples. In nonfasting samples, the mean difference was 0.125 mmol/L (−4.84 mg/dL) (95% CI −0.131, −0.120 mmol/L [−5.04, −4.64 mg/dL]).

Next, to determine the effect of postprandial time on LDL-C concentrations, we plotted the 2.5%, 25%, 50%, 75%, and 97.5% cumulative percentile and extreme values by 2-h intervals of time to last meal. With greater time elapsed since last meal, the median concentration of direct LDL-C was somewhat higher, but the overall distributions of LDL-C were not substantially different (Fig. 2). Similar results were found for LDL-C by Friedewald calculation (results not shown).

RELATIONSHIP OF BASELINE LDL-C WITH INCIDENT CVD

During a median follow-up of 11.4 years, a total of 945 first CVD events occurred in the women with known fasting/nonfasting status. Associations of LDL-C by Friedewald calculation and direct method with incident CVD were examined by quintile cutpoints as well as by 1-SD and 1 mmol/L (38.6 mg/dL) increments (Table 3). After adjustment for age, randomized treat-

Table 3. Associations of Friedewald and direct LDL cholesterol with cardiovascular disease events.

	Per 1-quintile increase					
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for linear trend[b]
Range						
Friedewald, fasting, mg/dL	<102.3	102.3–120.6	120.7–137.9	138.0–159.6	>159.6	
Direct, fasting, mg/dL	<97.8	97.8–115.5	115.6–132.2	132.3–153.9	>153.9	
Direct, nonfasting, mg/dL	<97.8	97.8–115.5	115.6–132.2	132.3–153.9	>153.9	
HR (95% CI)[a]						
Friedewald, fasting	1.00	1.21 (0.90–1.62)	1.40 (1.06–1.85)	1.65 (1.26–2.16)	1.93 (1.49–2.51)	<0.001
Direct, fasting	1.00	1.31 (0.97–1.77)	1.53 (1.15–2.05)	1.99 (1.51–2.63)	2.11 (1.60–2.78)	<0.001
Direct, nonfasting	1.00	0.96 (0.59–1.55)	1.25 (0.79–1.98)	1.16 (0.73–1.85)	1.20 (0.74–1.93)	0.33
	Per 1-SD increase	Per 1 mmol/L (38.6 mg/dL) increase	*P*			
HR (95% CI)						
Friedewald, fasting	1.22 (1.14–1.30)	1.24 (1.15–1.34)	<0.001			
Direct, fasting	1.23 (1.15–1.32)	1.26 (1.17–1.37)	<0.001			
Direct, nonfasting	1.03 (0.89–1.18)	1.03 (0.88–1.22)	0.72			

[a] HRs and 95% CIs were adjusted for age, randomized treatment assignment, smoking status, menopausal status, postmenopausal hormone use, blood pressure, diabetes, and body mass index.
[b] *P* value for linear trend was obtained using the median value for each quintile. Fasting was defined as ≥8 h from last meal.

ment assignment, smoking status, menopausal status, postmenopausal hormone use, blood pressure, diabetes, and body mass index, the fasting LDL-C value obtained by either method was significantly associated with incident CVD to a similar extent. The adjusted HR per 1-SD increment in fasting Friedewald LDL-C was 1.22 (95% CI 1.14–1.30), almost identical to the fasting direct LDL-C HR of 1.23 (95% CI 1.15–1.32). Similarly, when compared per 1 mmol/L (38.6 mg/dL) increments, the fasting HRs were essentially identical. Only fasting LDL-C was associated with CVD. Nonfasting direct LDL-C showed no association with CVD ($P = 0.72$), nor did nonfasting Friedewald LDL-C ($P = 0.92$).

Finally, the use of LDL-C clinical cut-points based on NCEP categories of risk in the fasting participants (n = 19 777), Friedewald and direct LDL-C concentrations, led to classification of participants into the same NCEP risk category in 79.3% (15 686 of 19 777) participants (Table 4). A total of 4088 participants (20.7%) differed by 1 NCEP category, with a large proportion (3599 of 4088 or 88.0%) of these participants being classified into a lower risk NCEP category by direct LDL-C compared with Friedewald calcula-

Table 4. Concordance of Friedewald and direct fasting LDL cholesterol for classifying participants into NCEP categories of risk, N = 19 777.

		N (%) of participants in NCEP categories by Friedewald fasting LDL cholesterol, mg/dL			
		<100	100–129	130–159	≥160
N (%) of participants in NCEP categories by fasting direct LDL cholesterol, mg/dL	<100	3287 (16.6)	1158 (5.9)	0	0
	100–129	173 (0.9)	5458 (27.6)	1518 (7.7)	1 (0.0)
	130–159	0	193 (1.0)	4148 (21.0)	923 (4.7)
	≥160	1 (0.0)	1 (0.0)	123 (0.6)	2793 (14.1)

tion. Only 3 participants differed by 2 or more NCEP categories.

Discussion

In this prospective study of 27 331 initially healthy women, mean baseline concentrations of fasting and nonfasting direct LDL-C were lower by approximately 0.13–0.26 mmol/L (5–10 mg/dL), respectively, compared with fasting Friedewald LDL-C. The direct method used in this study correlated highly with Friedewald calculation. In fasting samples, the association of direct LDL-C with incident CVD was nearly identical to LDL-C by Friedewald calculation. However, the lower LDL-C concentrations measured by this direct assay may misclassify a substantial proportion of individuals into a lower NCEP risk category. Moreover, the lack of association of nonfasting direct LDL-C with CVD calls into question the clinical utility of a direct assay for LDL-C measurement in a nonfasting blood sample.

Several direct methods for measuring LDL-C are currently available, *(10)*, but there are few data evaluating their predictive performance in relation to clinical events. Potential advantages of direct measurement of LDL-C are believed to be better precision of the assay owing to the single measurement and relative lack of effect of the presence of increased triglyceride concentrations or a nonfasting blood draw *(5)*. Some studies have shown direct assays to be generally accurate compared to the β-quantification reference method or the Friedewald calculation *(11–14)*. However, other studies have questioned the specificity of direct assays and their ability to meet the NCEP goal for a total error of $<$12% *(15)*. In addition, clinical trials demonstrating the benefit of LDL-C lowering with statin therapy have used Friedewald calculation for determining LDL-C concentration *(16)*, with the exception of the Heart Protection Study, which used a direct assay *(17)*. Our study findings demonstrate no clear advantage for using a direct assay for LDL-C compared with Friedewald calculation. Moreover, LDL-C concentration with the direct assay used in this study was approximately 0.13–0.26 mmol/L (5–10 mg/dL) lower than by Friedewald calculation. Although small, this systematic difference in mean LDL-C concentrations may be clinically important when NCEP risk categories are used to assess the need for drug intervention in a particular individual.

For nonfasting individuals, the direct assay has been suggested to be the preferred method for assessment of LDL-C *(1)*. The findings from this study call into question the clinical utility of performing a direct assay for LDL-C measurement in nonfasting samples, because nonfasting LDL-C by direct assay was not associated with CVD. By contrast, nonfasting levels of apolipoprotein B_{100} and the ratio of apolipoprotein B_{100}/A-1 were associated with CVD in this group of women, although the association with CVD events was stronger for data obtained from fasting samples *(18)*. Importantly, the ratio of total/HDL cholesterol was associated with CVD to a similar magnitude in both fasting and nonfasting samples and can be obtained at no additional cost *(18)*. Direct assays add to healthcare costs and are more expensive than measuring triglycerides, total cholesterol, or HDL cholesterol. Future studies are needed to assess the association with CVD of direct LDL-C assays in other populations, examined according to fasting status.

There are several possible limitations of the present study. Lipid measurements were available only once at baseline, and results could not be corrected for potential regression dilution bias. Although we assessed only the direct Roche method, this assay is commonly used and commercially available in the US. Our study included healthcare professionals who were women, mostly white, apparently healthy, and recruited from a variety of geographic locations across the US; thus, it is unclear if our results would be applicable to other ethnic populations or men. Time to last meal was self-reported, and we did not have paired samples of fasting and nonfasting measurements in the same individuals. Finally, this was a primary-prevention population, and further studies are needed before the data can be extended to secondary-prevention populations that are frequently treated with lipid-lowering medications.

Strengths of the present study include the large number of healthy women participants from whom simultaneous concentrations of direct and Friedewald LDL-C were obtained. Additionally, all lipid measurements were performed at a core laboratory facility that is certified for lipid testing by the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization program. Detailed information on cardiovascular risk factors was available, allowing for analysis by the presence or absence of these factors, such as fasting status.

In summary, the direct assay used in this study correlated highly with Friedewald calculation but was generally lower by approximately 0.13–0.26 mmol/L (5–10 mg/dL). The lower LDL-C concentrations by direct assay may misclassify a substantial proportion of individuals into a lower NCEP risk category. Although the association of LDL-C with CVD by the 2 methods was nearly identical in fasting samples, the lack of association of nonfasting direct LDL-C with CVD raises questions regarding the clinical use of a direct assay for LDL-C in nonfasting blood samples.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting*

or revising the article for intellectual content; and (c) final approval of the published article.

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