

Fasting and Nonfasting LDL Cholesterol: To Measure or Calculate?

Børge G. Nordestgaard* and Marianne Benn

Whether lipid profiles should be measured in the fasting or nonfasting state is a hot topic (1, 2). The fasting state is that used conventionally (3, 4); however, it would be much simpler for patients worldwide if a lipid profile could be taken at any time of the day, irrespective of the time since and the content of the last meal. In both the US and Europe, LDL cholesterol is currently considered the most important measurement in a lipid profile (3, 5).

Direct assays for measuring LDL cholesterol are widely available and used in many laboratories; however, even if LDL cholesterol measured with a direct method gives results similar to those calculated with the Friedewald equation, it is unclear how the 2 measurements compare in predicting ischemic cardiovascular disease. In this issue of *Clinical Chemistry*, Mora et al. report on an evaluation of fasting LDL cholesterol concentrations calculated with the Friedewald equation vs direct measurement of fasting and nonfasting LDL cholesterol concentrations for predicting cardiovascular disease in a prospective study of 27 331 women from the Women's Health Study (6). They also examined misclassification of individuals into the National Cholesterol Education Program risk categories (3) with direct measurement of LDL cholesterol, compared with conventional Friedewald calculation of LDL cholesterol. These topics are timely and important.

In 1972, Friedewald, Levy, and Fredrickson presented a new method for estimating LDL cholesterol and compared it with the gold standard of preparative ultracentrifugation (7). The method required only the measurement of plasma total cholesterol, HDL cholesterol, and triglyceride concentrations—all of which can be measured without the use of laborious and expensive ultracentrifugation. For this reason, the method fundamentally changed clinical practice for risk estimation, made possible epidemiologic studies that included LDL cholesterol as a risk predictor, and later provided a way to easily assess the adequacy of statin

treatment aimed at reducing LDL concentrations and cardiovascular disease.

The Friedewald equation calculates LDL cholesterol as total cholesterol minus VLDL cholesterol minus HDL cholesterol. According to the Friedewald calculation, LDL cholesterol includes intermediate-density lipoprotein (IDL) cholesterol and lipoprotein(a) cholesterol. VLDL cholesterol is calculated as triglycerides divided by a factor of 5 when lipids are measured in milligrams per deciliter and by a factor of 2.22 when measured in millimoles per liter. The triglycerides/5 ratio as a proxy for VLDL cholesterol is based on the observation that the ratio of the mass of triglycerides to that of cholesterol in VLDL is relatively constant in the fasting state, approximately 5:1 in healthy individuals (7); however, this means of estimating VLDL cholesterol introduces the well-known limitations of the Friedewald equation. First, at triglyceride concentrations >400 mg/dL or in the nonfasting state when chylomicrons, chylomicron remnants, or VLDL remnants are present, the triglyceride/cholesterol ratio in VLDL will be greater than 5:1, the VLDL cholesterol concentration will consequently be overestimated, and LDL cholesterol will therefore be underestimated. Second, in rare patients with type III hyperlipidemia (remnant hyperlipidemia, dysbetalipoproteinemia) in which cholesterol-rich β -VLDLs are present, the VLDL cholesterol concentration will be underestimated, and LDL cholesterol therefore will be overestimated (7).

During the 1990s, several methods for direct measurement of LDL cholesterol were introduced, but not until 1998, with the introduction of the homogeneous or third-generation assays, did direct measurement of LDL cholesterol become useful in routine clinical practice (8). The homogeneous assays directly measure LDL cholesterol after either blocking or solubilizing other lipoprotein classes. These assays are not or only mildly influenced by the presence of chylomicrons and chylomicron remnants and therefore theoretically should not be influenced by a nonfasting state. Direct homogeneous assays have limitations, however, including (a) varying specificity for the LDL cholesterol fraction, leading in general to underestimation of the LDL cholesterol concentration (87%–105% recovery of LDL cholesterol); (b) often including VLDL cholesterol in the LDL fraction; (c) only measuring 31%–

Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark.

* Address correspondence to this author at: Department of Clinical Biochemistry, Herlev Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark. Fax +45 44883311; e-mail brno@heh.regionh.dk.

Received February 20, 2009; accepted February 25, 2009.

Previously published online at DOI: 10.1373/clinchem.2008.123083

64% of the IDL; and (d) including lipoprotein(a) to a varying and often unknown degree (4, 8).

Mora et al. stratified these 2 alternatives (i.e., LDL cholesterol concentrations obtained indirectly via Friedewald calculations and direct homogeneous measurement of LDL cholesterol) for fasting status and compared them with respect to their utility to predict cardiovascular disease (6). Previous studies mainly focused on comparing the analytical precision and accuracy of the 2 methods (8), whereas the present report (6) is the first to also evaluate these alternatives' clinical usefulness and predictive ability for cardiovascular disease in a very large prospective study with 11 years of follow-up. The authors found that measured and calculated LDL cholesterol values were highly correlated, both in fasting individuals ($n = 19\,777$) and in nonfasting individuals ($n = 6615$) (both Pearson correlation coefficients were >0.97). In addition, a Bland-Altman bias plot revealed that the direct method produced values that were lower than calculated Friedewald values by 6 mg/dL (0.15 mmol/L) in the fasting state and by 5 mg/dL (0.13 mmol/L) in the nonfasting state. Consequently, the direct method classified a fifth of the participants into a lower National Cholesterol Education Program risk category than did the Friedewald calculation of LDL cholesterol; therefore, these individuals are less likely (incorrectly) to be given cholesterol-lowering therapy.

In fasting women, an increase of 1 SD of the directly measured LDL cholesterol concentration increased the risk of cardiovascular disease by 23%, and a corresponding increase in the SD of Friedewald LDL cholesterol concentration increased the risk similarly, by 22%. Surprisingly, neither nonfasting direct measurement of LDL cholesterol nor nonfasting Friedewald calculation of LDL cholesterol predicted risk of cardiovascular disease, a finding in accordance with a previous study from the Women's Health Study (2); however, in that study, which was based on the same individuals as the present study (2), only 207 cardiovascular events occurred among nonfasting women, whereas in a parallel study from the Copenhagen City Heart Study with 1166 cardiovascular events, an increased nonfasting LDL cholesterol concentration calculated according to Friedewald equation predicted an increased risk of cardiovascular disease in both women and men (1).

Recent studies have reported that increased concentrations of nonfasting triglycerides, a marker of increased concentrations of remnant cholesterol, are strongly associated with a higher risk for cardiovascular disease and early death in both women and men (9–11). This result supports the use of nonfasting rather than fasting lipid measurements. Also in favor of the use of nonfasting samples is the fact that lipid profiles change minimally at most in response to typical food

intakes (1, 2). On the basis of such evidence, hospitals in Copenhagen and elsewhere in Denmark now use nonfasting lipid profiles as the standard and suggest a repeat fasting triglyceride measurement only if nonfasting concentrations exceed 4 mmol/L (352 mg/dL).

The study by Mora et al. (6) has provided important insights, but we are nevertheless left with other important unanswered questions. Will the results be the same in men? Will the results change if fresh plasma is used for measurements rather than the frozen samples used in the Women's Health Study? Will the results be similar for direct LDL cholesterol assays different from the one used in the present study? How do Friedewald and direct LDL cholesterol methods compare at triglycerides >400 mg/dL (>4.52 mmol/L)? Because direct measurement of LDL cholesterol is clearly more expensive than the "free of charge" Friedewald calculation of LDL cholesterol, should the standard practice be to use the calculated Friedewald LDL cholesterol value if triglycerides are <400 mg/dL and to use direct assays only at higher triglyceride values? It is hoped that future studies can answer some of these questions.

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.

Authors' Disclosures of Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References

1. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. *Circulation* 2008;118:2047–56.
2. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation* 2008;118:993–1001.
3. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;285:2486–97.
4. Rifai N, Warnick GR. Lipids, lipoproteins, apolipoproteins, and other cardiovascular risk factors. In: Burtis C, Ashwood E, Bruns D, eds. *Tietz textbook of clinical chemistry and molecular diagnostics*. St. Louis: Elsevier Saunders; 2006. p 903–81.
5. Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, et al. European guidelines on cardiovascular disease prevention in clinical practice: executive summary. *Atherosclerosis* 2007;194:1–45.
6. Mora S, Rifai N, Buring JE, Ridker PM. Comparison of LDL cholesterol concentrations by Friedewald calculation and direct measurement in relation to cardiovascular events in 27 331 women. *Clin Chem* 2009;55:

- 888–94.
7. 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;18:499–502.
 8. Nauck M, Warrick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. *Clin Chem* 2002;48:236–54.
 9. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007;298:299–308.
 10. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007;298:309–16.
 11. Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, Nordestgaard BG. Nonfasting triglycerides and risk of ischemic stroke in the general population. *JAMA* 2008;300:2142–52.