Inaccuracy of Lipid Measurements with the Portable Cholestech L-D-X Analyzer in Patients with Hypercholesterolemia

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Background: Although total cholesterol concentrations measured by portable lipid analyzers have acceptable bias and precision in young and middle-aged adults, clinically relevant differences in HDL-cholesterol (HDL-C) and triglyceride values have been described. Furthermore, the accuracy of portable lipid analyzers in older hyperlipidemic individuals, who have a high incidence of coronary heart disease, has not been validated. This study determined the biases and variability in portable lipid measurements in older patients with hypercholesterolemia and related them to National Cholesterol Education Program Adult Treatment Panel III guidelines.

Methods: Participants were ≥70 years of age with fasting serum LDL-cholesterol (LDL-C) concentrations >1.40 g/L. Fasting fingerstick samples were analyzed on a Cholestech L-D-X desktop analyzer. Antecubital venous samples were analyzed in a proficiency-certified clinical laboratory.

Results: Portable measurements systematically overestimated triglycerides (0.296 g/L; P <0.001) and HDL-C (0.015 g/L; P = 0.026). LDL-C concentrations were underestimated (0.043 g/L; P = 0.046). Total and non-HDL cholesterol calculations based on the portable lipid device provided unbiased estimates, but wide variability was present. Significant variability in lipid determinations limited their clinical usefulness in individual patients, especially because 2 SD of the mean bias between the laboratory and the portable determinations of LDL-C and non-HDL cholesterol exceeded the 0.30 g/L cutoff that defines treatment targets in the current lipid guidelines.

Conclusions: Lipid values obtained from portable lipid analyzers may be useful for screening, but they should not be used to make clinical decisions regarding the diagnosis and management of dyslipidemia in individual patients.

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Use of portable lipid analyzers (PLAs) to measure serum lipid values has become more common, especially because recent technical developments permit rapid determination of all components of the standard lipid profile (1). In general, PLAs are accurate, with biases and coefficients of analytical variation that meet the National Cholesterol Education Program (NCEP) Lipid Standardization Panel guidelines for acceptable total cholesterol measurement (total analytic error <8.9%) (1–5). Accurate measurement of HDL-cholesterol (HDL-C) by PLAs has also been reported, although the variability in PLA measurements is higher than that obtained with laboratory-based methods (1, 5–7). Furthermore, a study using one of the most popular PLAs used in the US showed statistically significant biases for all components of the lipid panel and clinically relevant overestimation of triglycerides and HDL-C (5).

Although these studies generally have shown acceptable bias and precision in the populations studied, clinically relevant differences between measurements in individuals have been observed. This is an important distinction because individual laboratory values and their trends dictate patient diagnosis and treatment algorithms (8). Furthermore, most participants in previous studies

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1 Nonstandard abbreviations: PLA, portable lipid analyzer; NCEP, National Cholesterol Education Program; and HDL-C and LDL-C, HDL- and LDL-cholesterol, respectively.
were young and had relatively normal lipid profiles, rather than the older, hyperlipidemic individuals who account for a larger burden of coronary heart disease in the US (9). The purpose of this study was to assess the biases and variability of a popular PLA in older patients with hypercholesterolemia and to relate them to the NCEP Adult Treatment Panel III guidelines (8).

**Materials and Methods**

**PARTICIPANTS**

The Institutional Review Board at the University of Wisconsin Medical School approved the study. All participants provided informed consent before participation. Adults ≥70 years of age with fasting serum LDL-cholesterol (LDL-C) concentrations >1.40 g/L and triglycerides <4.00 g/L were recruited for possible enrollment in a study of lipid-lowering medications and antioxidant vitamins. Current users of tobacco-containing products, cyclosporine, or warfarin were excluded, as were individuals with abnormal liver function tests or creatine kinase concentrations. Participants were not allowed to take lipid-lowering medications or vitamin supplements for 4 weeks before testing.

**LABORATORY MEASUREMENTS**

All laboratory and PLA analyses were performed at room temperature. Participants fasted for at least 12 h before laboratory tests and rested sitting upright for 15 min before blood-drawing procedures. Participants’ hands were washed with hospital soap and rinsed thoroughly with water, then cleaned with alcohol swabs and allowed to dry. Using lancets, we collected fingerstick samples into heparin-coated capillary tubes (Cholestech L-D-X capillary tube). Blood was allowed to flow freely from the fingerstick into the capillary tube without milking of the finger. Samples were then dispensed immediately onto commercially available test cassettes for analysis in a Cholestech L-D-X desktop analyzer. One lot of reagent cassettes was used for all studies. The Cholestech L-D-X PLA measured total cholesterol, triglycerides, and HDL-C in fingerstick blood, after passing samples through a test cassette that removed cellular components. The principle of analysis is as follows: Plasma flowing to the right side of the cassette moves to the total cholesterol and triglyceride enzymatic and colorimetric reaction pads (10, 11). Plasma flowing to the left side of the cassette moves through a region containing dextran sulfate and magnesium acetate, which precipitates apolipoprotein B-containing lipoproteins. The filtrate then moves to the HDL-C colorimetric reaction pad (7). A daily optics check was performed on the single analyzer used for all studies. All samples were obtained by a clinical research nurse with a Master’s degree in nursing and extensive clinical and research experience who received focused training on proper use of the Cholestech L-D-X PLA.

Immediately afterward, venous samples were obtained from an antecubital vein with a tourniquet time of <2 min and dispensed into evacuated red-top glass collection tubes (Vacutainer; Becton Dickinson). After samples were centrifuged for 15 min at 1500g, 1.0-mL aliquots were withdrawn, and serum total cholesterol, HDL-C, and triglycerides were measured by automated enzymatic procedures on a Hitachi 747 analyzer with reagents from Roche, Inc. Serum total cholesterol was measured by a cholesterol ester–oxidase enzymatic procedure, which in our laboratory has a CV <1%. HDL-C was measured directly by an enzymatic colorimetric method that incorporated polyethylene glycol-modified cholesterol esterase. In our laboratory, this technique has a CV of 2–3%. Serum triglycerides were measured by a glycerol kinase-based enzymatic procedure that corrects for free glycerol. In our laboratory, this assay has a CV of 2–4%. LDL-C was estimated using the Friedewald formula (12). Non-HDL cholesterol was calculated as the difference between the total cholesterol and HDL-C concentrations (8, 13, 14). The Pacific Biometrics, College of American Pathologists, and State of Wisconsin Laboratory of Hygiene Proficiency Testing Programs have validated these procedures in our laboratory.

The University of Wisconsin Hospital 1998 College of American Pathologists proficiency testing survey results for all lipid measurements were compiled and compared with the CDC confirmatory data to establish possible biases with the laboratory methods using the Hitachi 747. Three challenges of five samples each provided 15 values for each analyte with which to establish biases from the proficiency testing data. Each analyte showed close agreement with the confirmatory values except for a slight but consistent underestimation of triglyceride values. The differences of the means (bias, laboratory minus CDC) for each analyte follow, with regression equations and correlation coefficients: total cholesterol = −0.006 g/L [−0.029 + 1.01(CDC); r = 0.996]; HDL-C = −0.003 g/L [0.010 + 0.97(CDC); r = 0.974]; triglycerides = −0.060 g/L [−0.023 + 0.96(CDC); r = 0.995]. For calculated values, the differences of the means and the equations are as follows: LDL-C = 0.007 g/L [−0.059 + 1.05(CDC); r = 0.993]; and non-HDL cholesterol = −0.003 g/L [−0.064 + 1.04(CDC); r = 0.996].

**STATISTICS**

All lipid values were described as the mean ± SD and range. Relationships between lipid values from the laboratory and PLA were described by linear regression and compared using paired Student t-tests. Agreement between lipid values obtained from the laboratory and the PLA were described as the mean bias (laboratory value minus PLA value) ± SD. This information was displayed graphically with the mean bias on the ordinate and laboratory lipid values displayed on the abscissa. Horizontal lines were drawn from the ordinate values representing the mean bias and the mean bias ± 2 SD (15).
NCEP, with the exception of triglycerides, for which the
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PLA (Table 1 and Fig. 2). The absolute bias in total cholesterol measurements by both techniques correlated modestly but with a large intercept (0.188 vs 0.024), but the differences between the laboratory and PLA total cholesterol values were not significant (P = 0.492; Table 1 and Fig. 2A). The PLA bias remained small (−0.010 g/L) after accounting for the small (0.006 g/L) non-concentration-dependent laboratory bias identified in proficiency testing. The SD of the (PLA − laboratory) bias was much larger than for the (laboratory − CDC) bias (0.147 vs 0.025 g/L). Accordingly, the (laboratory − CDC) intercept and slope were much smaller than analogous (PLA − laboratory) values.

HDL-C measurements by both techniques correlated strongly, but the intercept and slope were large, suggesting a methodologic error in PLA measurements (r = 0.96; y-intercept, −0.133 g/L; slope, 1.289; S_y|x = 0.041; Table 1 and Fig. 1B). The absolute bias in HDL-C measurements by the portable analyzer was small (−0.015 g/L), but the SD of the mean bias was large (0.051 g/L, or 9.9% of the mean HDL-C concentration). A strong concentration-dependent negative bias was identified (bias slope, −0.284), and the differences between the laboratory and PLA HDL-C values were significant (P = 0.026; Table 1 and Fig. 2B). The bias in HDL-C measurements by the PLA was not related to triglyceride concentrations (r = 0.03). The PLA bias remained small (−0.012 g/L) after accounting for the small (0.003 g/L) non-concentration-dependent laboratory bias identified in proficiency testing. The SD of the bias (PLA − laboratory) was much larger than for the (laboratory − CDC) bias (0.051 vs 0.017 g/L). Accordingly, the (laboratory − CDC) intercept and slope were much smaller than analogous (PLA − laboratory) values.

Triglyceride measurements by both techniques correlated strongly, but the intercept was large (r = 0.97; y-intercept, 0.329 g/L; slope, 0.979; S_y|x = 0.148; Table 1 and Fig. 1C). The absolute bias in triglyceride measurements by the PLA was large (−0.296 g/L), but the SD of the mean bias was not (0.147 g/L, or 9.4% of the mean triglyceride concentration). The bias was not concentration dependent (bias slope, −0.024), but the differences between the laboratory and PLA triglyceride values were significant (P < 0.001; Table 1 and Fig. 2C). The bias in triglyceride measurements by the PLA was not related to HDL-C concentrations (r = 0.01). The PLA bias remained large (−0.236 g/L) after accounting for the small (0.060 g/L) laboratory bias identified in proficiency testing. The SD of the (PLA − laboratory) bias was much larger than for the (laboratory − CDC) (0.147 vs 0.035 g/L, indicating scatter in the PLA method. Accordingly, the (laboratory − CDC) intercept and slope were much smaller than analogous (PLA − laboratory) values.

LDL-C calculations by both techniques correlated strongly with a large intercept (r = 0.84; y-intercept, 0.352 g/L; slope, 0.792; S_y|x = 0.157; Table 1 and Fig. 1D). The absolute bias in LDL-C measurements by the PLA was small (0.043 g/L), but the SD was large (0.169 g/L, or 8.9% of the mean LDL-C concentration). Two SD of the mean bias exceeded the 0.30 g/L cutoff that defines treatment targets in the NCEP guidelines (8). This cutoff was
exceeded by eight (12.7%) of the PLA LDL-C calculations, including three overestimations and five underestimations of laboratory LDL-C calculations. The bias was slightly concentration dependent (bias slope, \(-0.080\)). With the Friedewald formula for estimating LDL-C concentrations, the mean triglyceride value overestimation (0.296 g/L) would have been expected to produce an \(-0.060\) g/L overestimation of the VLDL-cholesterol concentration (Fig. 2D) (12). This would lead to a systematic underestimation of LDL-C values, which was close to the 0.043 g/L bias observed in this study. The remainder of the difference (0.017 g/L) was methodology and concentration dependent. The difference between the laboratory and PLA LDL-C values was significant \((P = 0.046; \text{Table } 1 \text{ and Fig. } 2B)\). The SD of the mean (PLA − laboratory) bias was much larger than for the (laboratory − CDC) bias (0.169 vs 0.035 g/L), indicating scatter in the PLA calculations. Accordingly, for calculated LDL-C, the laboratory − CDC intercept and slope were much smaller than analogous (PLA − laboratory) values.
Calculation of non-HDL cholesterol avoids assumptions inherent in the Friedewald formula (12–14). Non-HDL cholesterol calculations using lipid values from both methods correlated strongly but with a large intercept ($r_{\text{HA}} = 0.88$; $y$-intercept, 0.417 g/L; slope, 0.813; $S_{\text{HAx}}$ = 0.172; Table 1 and Fig. 1A). The absolute bias in non-HDL cholesterol calculations by the PLA was small ($-0.002$ g/L), and a slightly negative concentration-related bias was present (bias slope, $-0.053$; Table 1 and Fig. 2E). The SD of the bias was large (0.185 g/L, or 8.3% of the mean non-HDL cholesterol value), and 2 SD of the mean bias exceeded the 0.30 g/L cutoff that defines treatment targets in the NCEP guidelines (8). This cutoff was exceeded by eight (12.7%) PLA non-HDL cholesterol calculations, including four overestimations and four underestimations of laboratory non-HDL cholesterol calculations. Differences between the laboratory and PLA values, however, were not significant ($P = 0.946$) (8, 16, 17). The PLA bias

![Fig. 2. Bias plots of agreement between laboratory and PLA values.](image-url)

(A), total cholesterol. Bias = 0.190 – 0.75(portable cholesterol); $r = 0.15$; $S_{\text{HA}} = 0.370$; mean bias, $-0.016$ g/L; 2 SD = 0.376 g/L; $P = 0.492$. Chol, cholesterol. (B), HDL-C. Bias = 0.135 – 0.284(portable HDL-C); $r = 0.81$; $S_{\text{HA}} = 0.030$; mean bias, $-0.05$ g/L; 2 SD = 0.102 g/L; $P = 0.026$. (C), triglycerides. Bias = $-0.250$ – 0.024(portable triglycerides); $r = 0.11$; $S_{\text{HA}} = 0.147$; mean bias, $-0.296$ g/L; 2 SD = 0.294 g/L; $P < 0.001$. TG, triglycerides. (D), calculated LDL-C. Bias = 0.192 – 0.080(portable LDL-C); $r = 0.14$; $S_{\text{HA}} = 0.168$; mean bias, 0.043 g/L; 2 SD = 0.338 g/L; $P = 0.046$. (E), calculated non-HDL cholesterol. Bias = 0.116 – 0.053(portable non-HDL cholesterol); $r = 0.14$; $S_{\text{HA}} = 0.168$; mean bias, $-0.002$ g/L; 2 SD = 0.369 g/L; $P = 0.946$. - - - , bias; --- ---, 2 SD of the mean.
remained small (0.002 g/L) after accounting for the small (0.003 g/L) laboratory bias identified with values used in proficiency testing. The SD surrounding the (PLA − laboratory) bias was much higher than for (laboratory − CDC) bias (0.185 vs 0.030 g/L). Accordingly, the (laboratory − CDC) intercept and slope were much smaller than analogous (PLA − laboratory) values.

**Discussion**

This study represents the first cohort of hypercholesterolemic older adults assessed by a PLA. Clinically relevant variability in the measurement of all lipid values by the PLA was detected, but total cholesterol measurements from the PLA provided unbiased estimates that were not statistically different from laboratory measurements. In contrast, HDL-C and triglyceride measurements from the PLA significantly overestimated laboratory measurements despite strong, direct correlations. Accordingly, application of the Friedewald formula to values obtained from the PLA led to underestimation of calculated LDL-C values (12–14). Significant variability in lipid determinations limited their interpretation in individual patients, especially because 2 SD of the difference between the laboratory and the PLA determinations of LDL-C and non-HDL cholesterol was >0.30 g/L (15.9% and 13.5% of the study mean, respectively). This value corresponds to the difference between cutoffs for the diagnosis and treatment of patients with lipid abnormalities established by the recent NCEP Adult Treatment Panel III (8). Variability was even more dramatic for HDL-C. Clinically relevant cutoffs for making therapeutic decisions regarding HDL-C concentrations have not been described, but a 0.07 g/L (13.6% of the study mean) difference would generally be considered large. This value was exceeded by 15 (23.8%) of the PLA HDL-C values, including 10 overestimations and 5 underestimations of laboratory HDL-C values.

Accounting for the very small laboratory biases detected in proficiency testing programs did not affect the results. Indeed, the variability of the PLA method, reflected in the slopes of the regression lines and the SDs of the biases between the PLA and laboratory, were very high and in stark contrast to the strong agreement between the laboratory and CDC. In spite of the differences in the individual components of the lipid profile, which in part were related to assumptions inherent in the Friedewald formula, total and non-HDL cholesterol values were not significantly different when determined by the laboratory and the PLA. Nevertheless, although the biases of total and non-HDL cholesterol determinations appeared to be small, the scatter was wide and rendered the variability in non-HDL cholesterol measurements by the PLA unacceptable for clinical use in individual patients.

Non-HDL cholesterol, the difference between the total cholesterol and HDL-C concentrations, represents the concentration of cholesterol carried in atherogenic lipoproteins, including LDL, VLDL, intermediate-density lipoproteins, and lipoprotein(a) (8, 13, 14, 16, 17). Calculation of non-HDL cholesterol has been recommended as a useful way of overcoming limitations of the Friedewald formula in patients with hypertriglyceridemia and in patients with diabetes mellitus (8, 13, 14, 16, 17). Non-HDL cholesterol recently has been included in several guidelines, including those at the University of Wisconsin Hospital and Clinics and the Third Adult Treatment Panel of the NCEP (8, 16, 17). This study implies that use of the non-HDL cholesterol calculation may overcome some of the biases in the techniques used by PLAs; however, the variability was large. Lipid screening based on both total cholesterol and non-HDL cholesterol may be useful, but their individual components appear to be too inaccurate to be used for clinical risk assessment, initiation of therapy, and longer individual follow-up.

**Limitations**

Use of the tourniquet to block venous return can lead to loss of water and electrolytes from plasma to the extracellular space, producing increases of 5–8% in the concentration of plasma proteins (18). Tourniquet time was minimized to <2 min in this study, but this may account for a small component of the overestimation of laboratory values by the PLA observed in this study. Because all fingerstick samples were obtained before phlebotomy for venous samples, a systematic bias also may have been introduced, but this is likely to have been very small (5).

The most significant source of differences between techniques is the laboratory’s triglycerides assay, which accounts for the presence of free glycerol by a blanking technique. This difference is usually <0.100 g/L within the range of triglyceride concentrations measured in patients in this study. This would account for <0.020 g/L of the observed bias in LDL-C values, but it cannot be considered a deficiency in the PLA method. If the blanking technique were accounting for a large amount of the difference, however, the overestimation of laboratory triglyceride measurements by the PLA would have been expected to increase as triglyceride concentrations increased, when glycerol is more likely to be present in the serum. In this study, overestimation of triglycerides by the PLA remained constant across a wide range of triglyceride concentrations (0.40–3.82 g/L; Fig. 2C), indicating that the error in LDL-C measurements primarily was to overestimation of triglycerides and application of the Friedewald formula. Furthermore, hand lotions and skin treatments were carefully washed off with soap, water, and alcohol to avoid falsely increasing triglycerides when the fingerstick method was used.

Finally, an even more accurate assessment of error in the PLA method could have been obtained if subject samples were split and sent to a reference laboratory that used Abell–Kendall techniques to measure cholesterol. Given the very small biases and imprecision in our laboratory values compared with reference values, across
a wide range of lipid values, clinically relevant differences from our laboratory are very unlikely.

In conclusion, PLAs are easy to use and provide rapid determination of lipid values in patients with hypercholesterolemia. Portable measurements of triglycerides and HDL-C were overestimated systematically, and LDL-C was underestimated. Unacceptable variability also was present. Total cholesterol and non-HDL cholesterol calculations based on results obtained by the portable lipid device provided unbiased estimates, but wide variability was present. Lipid values obtained from PLAs may be useful for screening, but they should not be used to make clinical decisions regarding the diagnosis and management of dyslipidemia in individual patients. Use of the total cholesterol and non-HDL cholesterol values obtained from PLAs warrants further investigation in larger trials.

References