correspondence

The Plasma Small Dense LDL-Cholesterol Calculation Formula Proposed by Srisawasdi et al Is Not Applicable to Koreans Who Are Healthy or Have Metabolic Syndrome

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To the Editor

We read with great interest the article by Srisawasdi et al[1] regarding a new formula for the estimation of small, dense, low-density lipoprotein cholesterol (sdLDL-C) levels. The investigators studied the correlation between classic lipid measures and sdLDL-C measured directly via a newly developed homogeneous method, in a total of 220 subjects without liver or renal disease. Using the data, they deduced the following simple equation for calculating sdLDL-C:

$$\text{sdLDL-C}_{\text{mg/dL}} = 0.580\ (\text{non–HDL-C}) + 0.407\ (\text{dLDL-C}) - 0.719\ (\text{cLDL-C}) - 12.05$$

where cLDL-C indicates calculated low-density lipoprotein cholesterol; dLDL-C, direct low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol.

According to their results, the coefficient of determination (R^2) between directly measured sdLDL-C and the calculated value was 0.878 while their mean difference and SD of the difference were only 0.85 mg/dL (0.02 mmol/L) and 9.2 mg/dL (0.24 mmol/L), respectively.[1] Further, they indicated that this formula provides a good estimate of sdLDL-C levels in healthy Thai individuals regardless of differences in sex, age group, creatinine level, or state of glucose intolerance.

Prompted by the article by Srisawasdi et al,[1] we conducted a validation study of the sdLDL-C equation in healthy Koreans and in patients with metabolic syndrome (MetS) to determine whether it can be applied to the Korean population in general and to a specific patient group with abnormal lipoprotein metabolism. A total of 318 Korean subjects (163 men and 155 women) with MetS were randomly selected from the Korean Metabolic Syndrome Research Initiatives (KMSRI)–Seoul cohort study.[2] A total of 360 age- and sex-matched healthy controls (181 men and 179 women) were also randomly selected from the same cohort. We excluded subjects younger than 20 years or older than 70 years of age, those with a history of familial lipid disorders or dyslipidemia-related diseases, as well as individuals with renal disease, hepatic disease, infectious diseases, or malignancy. MetS was diagnosed according to the National Cholesterol Education Program Adult Treatment Panel definition of metabolic syndrome modified for the Asian population.[3] All subjects completed a lifestyle questionnaire and anthropometric survey, and all biochemical samples were collected in accordance with the study protocol approved by the institutional review board of Severance Hospital, Yonsei University, Seoul, Korea.

We determined sdLDL-C levels using thawed serum samples stored frozen below –70°C. Serum sdLDL-C levels were measured using a homogeneous enzymatic assay (Denka Seiken, Tokyo, Japan) on a Hitachi-7600 analyzer (Hitachi, Tokyo, Japan). The Randox reagents used by Srisawasdi et al are essentially the same as the Denka Seiken reagents; however, they used a different instrument (Dimension RxL Max, Siemens Medical Solution Diagnostics, Tarrytown, NJ).[1] None of the patients enrolled in the present study had triglyceride levels higher than 400 mg/dL (4.52 mmol/L), and the results of a comparison between directly measured sdLDL-C and calculated sdLDL-C levels according to subgroup are shown **Table 1**. We used PASW Statistics software version 18.0.0 (SPSS, Chicago, IL) for correlation and multiple linear regression analysis. We also used SAS version 9.1.3 (SAS Institute, Cary, NC) for comparison of Pearson correlation or regression coefficients.

In the overall study population, the equation slightly overestimated the sdLDL-C level (mean difference, 3.11 ± 0.43 mg/dL). The R^2 value corresponding to the relationship between the directly measured sdLDL-C values and those computed using the proposed equation was 0.629, which is much lower than the R^2 of 0.878 reported by Srisawasdi et al.[1] The lower explanatory power of the equation may be related to basic differences in study designs such as the ethnicity of the study populations (Thai vs Korean) or the lipid measurement methods (Siemens vs Roche for triglycerides; Siemens vs Sekisui for total cholesterol, HDL-C, and LDL-C). Srisawasdi et al claimed that the lipid test showed acceptable performance according to the Lipid Standardization Program (LSP) developed by the Centers for Disease Control and Prevention (Atlanta, GA). Although our laboratory does not participate in LSP, we are enrolled in the College of

Table 1
Regression Analysis Between Measured and Calculated sdLDL-C* Values According to Subgroups

Group	n	*r*	SE, mg/dL	Bias, mg/dL	Coefficients (95% Confidence Interval)	
					Slope	Intercept
Sex						
Male	344	0.711	11.385	2.46	0.630 (0.564-0.697)	18.290 (15.204-21.375)
Female	334	0.877	6.877	3.78	0.730 (0.687-0.773)	13.415 (11.709-15.121)
P		<.0001†		.1208‡	.0159§	.0058§
Disease status						
Healthy	360	0.809	7.575	3.28	0.669 (0.619-0.720)	13.953 (12.143-15.762)
MetS	318	0.688	10.646	2.92	0.577 (0.510-0.645)	22.896 (19.497-26.295)
P		.0003†		.6805‡	.0324§	<.0001§
All subjects	678	0.793	9.477	3.11	0.682 (0.642-0.722)	15.608 (13.897-17.319)

MetS, metabolic syndrome; sdLDL-C, small, dense, low-density lipoprotein-cholesterol; SE, standard error of estimate.
* sdLDL-C calculated according to the formula proposed by Srisawasdi et al.[1]
† Pearson correlation coefficient difference of either sex or disease status subgroups using Fisher *z* transformation.
‡ Student *t* test.
§ Regression coefficient (slope and intercept) difference of either sex or disease status subgroup using *t* test.

American Pathologists Accuracy-Based Lipids Survey and our method demonstrated acceptable performance according to their standards.

The equation also overestimated sdLDL-C levels in both the healthy group (3.28 ± 0.48 mg/dL [0.08 ± 0.01 mmol/L]) and the MetS group (2.92 ± 0.73 mg/dL [0.07 ± 0.01 mmol/L), and the biases in each group were not significantly different (Table 1). Although the correlation between the directly measured and calculated sdLDL-C values was significant in both groups (healthy group, $r = 0.809$, $P < .001$; MetS group, $r = 0.688$, $P < .001$), the slope and offset were quite different ($P = .0324$ and $P < .0001$, respectively), and R^2 was better in the control group (0.654 vs 0.473, $P = .0003$), as shown in Table 1. This implies that the proposed equation may not be suitable for the calculation of sdLDL-C in Korean patients with MetS.

We performed multiple linear regression analysis using lipid profiles as the only independent variables for each disease group to generate a formula based on our data. In the healthy control group, dLDL-C showed collinearity (variance inflation factor >10) with both cLDL-C and total cholesterol, and the correlation coefficient value of dLDL-C with sdLDL-C was not significant. We therefore excluded dLDL-C from the regression analysis and obtained the following equations:

$$\text{sdLDL-C}_{\text{mg/dL}} = 0.690\,[\text{non–HDL-C}] - 0.410\,[\text{cLDL-C}] - 15.276,\ R^2 = 0.714$$

$$\text{sdLDL-C}_{\text{mg/dL}} = 0.084\,[\text{TG}] + 0.281\,[\text{TC}] - 0.251\,[\text{HDL-C}] - 17.236,\ R^2 = 0.714$$

where TC indicates total cholesterol and TG, triglyceride.

In patients with MetS, the aforementioned collinearity between cLDL-C, TC, and dLDL-C was not found, and the equation deduced for this group showed a lower R^2 of 0.640. The R^2 value for the overall study population was 0.711.

Although we achieved a better R^2 value by generating a linear equation from our own data, the observed R^2 value was still inferior to that reported by Srisawasdi et al.[1] However, the slightly better correlation detected between directly measured and calculated sdLDL-C levels in women compared with men (Table 1) may have played a role in the difference between our study and that of Srisawasdi et al because their gender ratios were different from ours.

Our findings suggest that the equation proposed by Srisawasdi et al[1] is not directly applicable to the Korean population. Furthermore, the performance of the proposed equation was worse in the MetS group compared with the healthy group, with a significant difference between calculated and directly measured values (Table 1), which obviates its generalization to patients with MetS. Therefore, to determine a generally applicable formula for the estimation of sdLDL-C, the correlation between conventional lipid parameters and sdLDL-C should be extensively evaluated across different ethnic and disease groups. In addition, standardized methods should be used for conventional lipid measurement.

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The Authors' Reply

We thank Dr Cho and colleagues for their interesting validation study on our formula for the estimation of small, dense, low-density lipoprotein cholesterol (sdLDL-C) in a Korean population. They found that our equation was not directly applicable to a Korean population because of overestimation of sdLDL-C in both healthy subjects and patients with metabolic syndrome.

Several reasons may be responsible for the disagreement between our study and the study by Cho et al. First, as we mentioned in our article, the variation in the LDL-C results may have contributed to the variation in calculated sdLDL-C. The principle of the LDL-C method used in our study (Siemens Medical Solution Diagnostics, Tarrytown, NY) was liquid selective detergent method that may differ from the Sekisui LDL-C assay (Sekisui Medical, Tokyo, Japan) used by Cho et al, which was based on selective solubilization using differences in affinity between lipoproteins and detergents. Because of the difference in methods, their results are not comparable. Second, the stability of sdLDL-C in samples should be of concern. Although Ito et al[1] found that the sdLDL-C particles in serum samples were stable for at least 4 days in a refrigerator, we did not know the stability of sdLDL-C in the frozen sample. In the Korean Metabolic Syndrome Research Initiatives (KMSRI)-Seoul Study,[2] the study samples from this cohort may have been stored at temperatures below –70°C since 2006 to 2007. Using frozen samples may have introduced a matrix effect on their results. Natural aggregation of lipoproteins may have occurred in samples stored for too long, which may have a bigger effect on sdLDL-C than on other lipoprotein. Using inappropriate samples might provide the low measured sdLDL-C concentration. Lastly, Cho et al introduced selection bias in their patient selection, with an inadequate distribution in lipid values. According to the KMSRI-Seoul Study,[2] we found that the range of total cholesterol and LDL-C levels in Korean samples may be lower and tighter than those in our study; (235.7 ± 76.9 mg/dL [6.1 ± 2 mmol/L] vs 195.9 ± 37.8 mg/dL [5.0 ± 0.97 mmol/L] for total cholesterol and 158.8 ± 72.7 mg/dL [4.1 ± 1.9 mmol/L] vs 117.8 ± 31.6 mg/dL [3.0 ± 0.81 mmol/L] for LDL-C). It is implied that mean sdLDL-C concentrations obtained in the KMSRI-Seoul study were not only lower than ours, but the spread of our values was much greater than in their study. This condition by itself may have resulted in differences in the results obtained via linear regression, but it also had a major effect on the correlation coefficient obtained in their study. If the tightness in the range of results is typical for the population being screened in Korea, their comments may be of particular interest; however, we would suggest that they investigate a broader range of values.

We absolutely agree that our proposed equation should be used across different ethnic and disease groups and also with different LDL-C methods.

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APTT Reagents for Different Coagulation Tests: One Size Does Not Fit All

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To the Editor

We read with interest the report by Fritsma et al[1] on the recommendations to use more than 1 reagent to screen for lupus anticoagulant (LA) in a hemostasis laboratory. Activated partial thromboplastin time (APTT) reagents supplied by different commercial sources are becoming increasingly specific not only for the presence of LA but also for different levels and types of coagulation factors and heparin.[2,3] The different factors affecting the sensitivity of these reagents are the type and concentration of phospholipids, type of activators, and the procedures followed in their preparation. Moreover, no data exist on the sensitivity of these reagents to various other clinical conditions associated with prolongation of APTT, like hypoalbuminemia, presence of active coagulation factors in circulation, microparticles, and monoclonal gammopathy.

To add some more information to that published by Fritsma et al, we advise that the 2 APTT reagents recommended for screening of LA should be from the same manufacturer. The diagnosis of LA is suspected mainly based on the difference in clotting times between low and high phospholipid reagents. Because different manufacturers use different concentrations of phospholipids, lupus-sensitive and lupus-insensitive reagents from different sources can give negative results on some occasions, even in the presence of LA. As rightly pointed out by our colleagues, a laboratory needs to have a good screening test for LA to avoid the use of more expensive confirmatory tests.

Another important point to mention here is the selection of APTT reagents for screening of factor VIII inhibitors. One of the major issues in measurement of these inhibitors with the conventional Bethesda assay or the modified assays is LA interference in a small percentage of patients with hemophilia.[4-6] So ideally lupus-insensitive APTT reagents should be used for detecting factor VIII or IX inhibitors to differentiate between LA and specific inhibitors to coagulation factors.

With more studies reporting the need for a clinical laboratory to use multiple APTT reagents, the question arises as to how many reagents should be procured to provide accurate results for the common coagulation tests. It is prudent to keep at least 2 types of APTT reagents; for routine factor assays and inhibitor assays, a lupus-insensitive reagent should preferentially be used. In patients receiving heparin therapy, LA-insensitive APTT reagents should be used. In patients clinically suspected of lupus inhibitors, LA-sensitive APTT reagents should be used. However while reporting such results, the clinical history of patients also should be integrated with this report, and remarks should include possible use of other tests clarifying prolonged APTT without apparent reason when mixing studies have produced either no or incomplete corrections.

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The Authors' Reply

We thank Drs Shetty and Ghosh for their comments. The development of activated partial thromboplastin time (APTT) reagent is complex and requires manufacturers to balance the effects of phospholipids, particulate activators, and matrix. The APTT reagent selection process at the local laboratory must weigh the needs of their patient population, including concerns about the prevalence of lupus anticoagulant (LA), possibility of coagulopathies, the

potential for encountering specific inhibitors, and the extent and types of anticoagulants (such as heparin and direct thrombin inhibitors) used at the institution. The prevalence of low-molecular-weight heparin therapy among outpatients may also be considered. Like Drs Shetty and Ghosh, we recommend an LA-insensitive reagent when the purpose of the APTT is primarily to monitor heparin therapy. We note also that the selection and application of reagents for coagulopathy screening, anticoagulation monitoring, and LA detection is a worldwide concern.

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