

Lipoprotein-Associated Phospholipase A₂, High-Sensitivity C-Reactive Protein, and Risk for Incident Ischemic Stroke in Middle-aged Men and Women in the Atherosclerosis Risk in Communities (ARIC) Study

Christie M. Ballantyne, MD; Ron C. Hoogeveen, PhD; Heejung Bang, PhD; Josef Coresh, MD, PhD; Aaron R. Folsom, MD, MPH; Lloyd E. Chambless, PhD; Merle Myerson, MD, EdD; Kenneth K. Wu, MD, PhD; A. Richey Sharrett, MD, DrPH; Eric Boerwinkle, PhD

Background: Measurement of inflammatory markers has been reported to identify individuals at increased risk for ischemic stroke. Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is a proinflammatory enzyme secreted by macrophages. We assessed Lp-PLA₂ and C-reactive protein (CRP) levels along with traditional risk factors to examine their relation to ischemic stroke.

Methods: A proportional hazards model was used in a prospective case-cohort study of 12 762 apparently healthy middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study who were observed for about 6 years.

Results: Mean Lp-PLA₂ and CRP levels adjusted for sex, race, and age were higher in the 194 stroke cases than the 766 noncases, whereas low-density lipoprotein cholesterol (LDL-C) level was not significantly different. Both Lp-PLA₂ and CRP levels were associated with ischemic stroke

after adjustment for age, sex, and race: hazard ratios were 2.23 for the highest vs the lowest tertile of Lp-PLA₂ and 2.70 for CRP level higher than 3 vs lower than 1 mg/L. In a model that included smoking, systolic hypertension, lipid levels, and diabetes, Lp-PLA₂ and CRP levels in the highest category were associated with hazard ratios of 1.91 (95% confidence interval, 1.15-3.18; *P* = .01) and 1.87 (95% confidence interval, 1.13-3.10; *P* = .02), respectively. Individuals with high levels of both CRP and Lp-PLA₂ were at the highest risk after adjusting for traditional risk factors compared with individuals with low levels of both, whereas others were at intermediate risk.

Conclusion: Levels of Lp-PLA₂ and CRP may be complementary beyond traditional risk factors in identifying middle-aged individuals at increased risk for ischemic stroke.

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AN ESTIMATED 700 000 strokes occur each year in the United States,¹ making stroke the third leading cause of death and the leading cause of neurologic disability. Although the incidence of stroke increases with age and is highest in the elderly, almost a third of strokes occur in individuals younger than 65 years.² Pharmacologic therapy with aspirin, antihypertensive medications, and statins are beneficial in the prevention of stroke; however, current US guidelines for screening and prevention of

C) and high-density lipoprotein cholesterol (HDL-C) are strongly associated with development of CHD in middle-aged Americans,^{3,4} and lipid screening should improve risk assessment for prevention of CHD, lipid levels are not highly predictive of stroke in middle-aged Americans.⁵

*See also pages 2454
and 2473*

Inflammation is postulated to play an important role in cerebrovascular disease as well as CHD, and levels of C-reactive protein (CRP) are associated with increased risk for stroke.^{4,6,7} Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is a proinflammatory enzyme secreted by macrophages that is primarily bound to LDL in the circulation. It hydrolyzes oxidized phospholipids to generate lysophosphatidylcholine and oxidized fatty acids, which have proinflammatory properties, and its activity is increased in small, dense LDL.⁸

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cardiovascular disease as recommended by the National Cholesterol Education Program Adult Treatment Panel III (ATP III) are based on the risk for development of coronary heart disease (CHD) and do not include risk for stroke. Although lipid fractions such as low-density lipoprotein cholesterol (LDL-

Author Affiliations are listed at the end of this article.

In this study, levels of Lp-PLA₂ and CRP were examined in a large population of middle-aged Americans in the Atherosclerosis Risk in Communities (ARIC) study⁹ to determine whether these inflammatory markers were associated with increased risk for incident ischemic stroke.

METHODS

STUDY POPULATION

The ARIC design, objectives, sampling strategies, and examination techniques have been described previously.⁹ The ARIC study is a large biracial cohort study of 15 792 adults aged 45 to 64 years. A baseline examination was conducted in the 1987-1989 period, and 3 more examinations were conducted through 1998.

STUDY DESIGN

Because plasma samples from the first visit were depleted, Lp-PLA₂ and high-sensitivity (hs)-CRP levels were measured in duplicate in plasma from visit 2 (1990-1992) in individuals who subsequently had an ischemic stroke (cases) and in a cohort random sample (CRS). Participants were excluded from follow-up if they did not return for visit 2 (n=1214), had a history of stroke at visit 2 (n=442), had prevalent CHD before visit 2 or missing CHD information (n=1272), belonged to an underrepresented minority group (n=91), or had no valid follow-up time (n=11). Therefore, the potential full cohort consisted of 12 762 individuals who were observed for incident ischemic stroke, a first definite or probable hospitalized stroke in a participant who did not have a history of physician-diagnosed stroke at visit 2. Criteria and classification for stroke have been described previously.¹⁰

A case-cohort design¹¹ (n=1225) was used to compare participants who developed a stroke with a CRS of all participants at the beginning of follow-up. The CRS was selected by stratification on sex, race (black vs white), and age at visit 2 (≥ 55 vs < 55 years). After exclusion of 265 subjects with missing information, the final sample size for the analysis was 960 (194 cases and 766 noncases). The CRS included 784 individuals (18 cases and 766 noncases).

RISK FACTOR ASSESSMENT

Medical history, cigarette smoking, and alcohol consumption were determined by standardized, validated, interviewer-administered questionnaires at visit 2. Body mass index (BMI) (calculated as weight in kilograms divided by the square of height in meters) was calculated from measured height at visit 1 and weight at visit 2. Waist-hip ratio was calculated from measurements obtained at visit 2. Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or use of antihypertensive medication in the past 2 weeks. Diabetes was defined as a fasting blood glucose level of 126 mg/dL (7.0 mmol/L) or higher, non-fasting blood glucose level of 200 mg/dL (11.1 mmol/L) or higher, a physician's diagnosis of diabetes, or use of antidiabetic medication in the past 2 weeks.

LABORATORY MEASUREMENTS

Plasma lipid levels were measured in centralized laboratories by standard, validated methods as previously described.¹²⁻¹⁴ We assessed Lp-PLA₂ levels by a dual monoclonal antibody immunoassay standardized to recombinant Lp-PLA₂ (PLAC; diaDexus Inc, South San Francisco, Calif).¹⁵ Interassay preci-

sion for Lp-PLA₂ measurement was assessed by measuring 2 controls of known concentration (low and high) in 40 separate assays; the interassay coefficients of variation on all 40 plates were 12.7% and 9.6%, respectively. We assessed CRP levels by the immunoturbidimetric CRP-Latex (II) high-sensitivity assay from Denka Seiken (Tokyo, Japan) performed according to the manufacturer's protocol and using a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, Ind). This assay has been validated against the Dade Behring method (Deerfield, Ill).¹⁶ For quality control, each sample was measured in duplicate, and about 6% of samples were measured as blinded replicates on different dates to assess repeatability of measurements of levels of Lp-PLA₂, CRP, and other analytes. The reliability coefficient for blinded quality control replicates was 0.76 for the Lp-PLA₂ assay (67 blinded replicates) and 0.95 for the CRP assay (70 blinded replicates).

STATISTICAL ANALYSIS

For the primary analysis, variables were categorized, using cut points from the ATP III guidelines¹⁷ for cholesterol and the US Joint National Committee VI guidelines¹⁸ for blood pressure (the guidelines in effect when this analysis was designed). Some categories were combined to maintain sufficient numbers of events per cell. For Lp-PLA₂, the major study covariate, tertiles were used. For hs-CRP, both tertiles and cut points from Pearson et al¹⁹ (hereinafter, Centers for Disease Control and Prevention/American Heart Association [CDC/AHA] guidelines) were examined. The primary null hypothesis was that Lp-PLA₂ level is not predictive of stroke beyond traditional risk factors, including consideration of the interrelationship of Lp-PLA₂ level with LDL-C and hs-CRP levels, as suggested in our research team's previous study of CHD.²⁰ A Wald test for the 2-sided alternative was used to test the association at the .05 level.

Means or proportions of baseline variables were examined in incident stroke cases vs noncases by analysis of covariance (adjusting for key demographic factors such as age, sex, and race whenever pertinent) and logistic regression, respectively.²¹ Basic demographic variables and risk factors included in the ATP III algorithm for CHD risk assessment,¹⁷ and thus chosen on an a priori basis, were considered as potential confounders, including LDL-C, HDL-C, and total cholesterol levels, diabetes, smoking, and hypertension. Weighted Pearson and Spearman rank correlation coefficients between variables were calculated for subjects in the CRS. In all analyses, weights based on sampling proportions were used to allow inferences to the entire cohort.

The Cox proportional hazards model was used to examine the independent and joint associations of Lp-PLA₂ level with incident ischemic stroke. The statistical method and computer software for case-cohort design within a framework of proportional hazard regression were modified to take into consideration the stratified nature of the CRS and robust variance estimation.^{22,23} The results are presented as hazard ratios (HRs) with 95% confidence intervals (CIs).

As a secondary analysis, tests were performed to assess for various potential interactions and (non)linearity, and subgroup analyses were conducted to confirm results. A χ^2 test was used to assess the overall association of Lp-PLA₂ level and outcome; all other overall associations were tested similarly. All statistical analyses were performed using SAS software, version 8 (SAS Institute Inc, Cary, NC), and SUDAAN version 8.0.0 (Research Triangle Institute, Research Triangle Park, NC).

RESULTS

Of the 223 incident strokes, 194 were classified as ischemic strokes, with mean time to ischemic stroke 4.4 years.

Table 1. Baseline Characteristics in Cases of Incident Ischemic Stroke and Noncases*

Characteristic	Incident Stroke (n = 194)	Noncases (n = 766)	P Value
Age, y†	59.7	56.8	<.001
Female, %†	43.8	57.6	<.001
African American, %†	43.3	24.3	<.001
Current smoker, %	33.7	19.7	<.001
BMI	28.3	28.1	.59
Diabetes, %	31.0	16.0	<.001
Hypertension history, %	58.9	32.6	<.001
Systolic blood pressure, mm Hg	129.6	121.5	<.001
Diastolic blood pressure, mm Hg	75.0	72.5	.01
Total cholesterol, mg/dL	212.6	208.0	.19
Triglycerides, mg/dL	147.3	126.1	<.001
HDL-C, mg/dL	46.6	50.8	<.001
LDL-C, mg/dL‡	136.6	132.0	.17
Lp-PLA ₂ , µg/L	443	374	<.001
hs-CRP, mg/L§	3.85	3.08	.01

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; Lp-PLA₂, lipoprotein-associated phospholipase A₂.

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

*Data are reported as mean values unless otherwise noted.

†Unadjusted mean or proportion; all others are adjusted for age, sex, and race.

‡Median LDL-C level was 136.2 mg/dL for cases and 131.6 mg/dL for noncases.

§Median hs-CRP level was 3.07 mg/L for cases and 1.76 mg/L for noncases.

Baseline characteristics of the study population are summarized in **Table 1**. Traditional stroke risk factors such as hypertension, diabetes, and current smoking were more prevalent in stroke cases. Compared with noncases, individuals with incident stroke had significantly higher systolic and diastolic blood pressure, higher triglyceride levels, and lower HDL-C levels than noncases. There was no significant difference in LDL-C levels between cases and noncases (136.6 vs 132.0 mg/dL [3.53 vs 3.41 mmol/L]). In addition to these differences in traditional risk factors, the weighted mean levels of both Lp-PLA₂ (443 vs 374 µg/L) and CRP (3.85 vs 3.08 mg/L) were significantly higher in cases than in noncases. There was no significant correlation between Lp-PLA₂ and CRP levels, but in the CRS, Lp-PLA₂ level was positively associated with LDL-C ($r=0.36$) and negatively associated with HDL-C ($r=-0.33$) levels, as previously reported.²⁰

In a Cox proportional hazards model adjusted for age, sex, and race, high CRP level, as defined by the CDC/AHA cut point of >3.0 mg/L was associated with a significant increase in risk (HR, 2.70; 95% CI, 1.69-4.31) (**Table 2**). After further adjustment for smoking status, systolic blood pressure, LDL-C and HDL-C levels, and diabetes, the risk associated with high CRP level remained significantly increased (HR, 1.87; 95% CI, 1.13-3.10) (**Table 3**). Levels of Lp-PLA₂ in the highest tertile (≥ 422 µg/L) were associated with an increased risk for incident ischemic stroke (HR, 2.23; 95% CI, 1.48-

Table 2. Ischemic Stroke Risk by CDC/AHA¹⁹ High-Sensitivity C-Reactive Protein Categories*

Model No.	Average Risk (1.0-3.0 mg/L)		High Risk (>3.0 mg/L)	
	HR (95% CI)	P Value	HR (95% CI)	P Value
1†	1.65 (1.02-2.67)	.04	2.70 (1.69-4.31)	<.001
2‡	1.41 (0.85-2.35)	.19	1.87 (1.13-3.10)	.02
3§	1.54 (0.91-2.62)	.11	1.97 (1.14-3.39)	.02

Abbreviations: ARIC, Atherosclerosis Risk in Communities Study; CDC/AHA, Centers for Disease Control and Prevention/American Heart Association; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio.

*The CDC/AHA low-risk category (<1 mg/L) is the referent; ARIC tertiles were lower than 1.01 mg/L, 1.01 to 2.82 mg/L, and higher than 2.82 mg/L.

†Adjusted for age, sex, and race.

‡Adjusted for age, sex, race, smoking status, systolic blood pressure, LDL-C and HDL-C levels, and diabetes.

§Adjusted for age, sex, race, smoking status, systolic blood pressure, LDL-C and HDL-C levels, diabetes, antihypertensive medication, and body mass index.

3.34) in a model adjusted for age, sex, and race. In a Cox proportional hazards model also adjusted for traditional risk factors of smoking status, systolic blood pressure, LDL-C and HDL-C levels, and diabetes, high levels of Lp-PLA₂ were still associated with a significant increase in risk for incident ischemic stroke (HR, 1.91; 95% CI, 1.15-3.18), and this association remained significant if CRP was included in the model (HR, 1.97; 95% CI, 1.16-3.33). Addition of antihypertensive medication and BMI to the model also did not change the results (HR, 1.93; 95% CI, 1.14-3.27); results were similar when the waist-hip ratio was substituted for BMI. Hazard ratios were similar for all incident stroke (data not shown). In the fully adjusted model, blood pressure, diabetes, current smoking, age, and race were all significant predictors of ischemic stroke, whereas LDL-C and HDL-C levels and sex were not.

Tests for potential interaction with variables used in the model did not show an interaction with race or sex, but a significant interaction was observed between Lp-PLA₂ and CRP levels ($P=.03$ for χ^2_4 test). Therefore, we examined the HR for ischemic stroke when both Lp-PLA₂ and CRP levels were included along with traditional risk factors in the same Cox proportional hazard regression model. The overall joint effect of Lp-PLA₂ and CRP levels was highly significant ($P<.001$ for χ^2_8 test), and as shown in the **Figure**, the effect of Lp-PLA₂ and CRP levels varied by the level of the other factor. Individuals with CRP levels lower than 1.0 mg/L and Lp-PLA₂ levels in the lowest tertile (<310 µg/L) were at the lowest risk; individuals with both a high level of CRP (>3.0 mg/L) and a high level of Lp-PLA₂ were at the highest risk (HR, 11.38; 95% CI, 3.13-41.41); and other groups were at an intermediate risk. The absolute 10-year risk for ischemic stroke in individuals with low levels of both Lp-PLA₂ and CRP was 0.4% vs an absolute risk of 5.8% in individuals with high levels of both Lp-PLA₂ and CRP.

Table 3. Ischemic Stroke Risk by Lipoprotein-Associated Phospholipase A₂ Tertiles*

Model No.	Tertile 2 (310-422 µg/L)		Tertile 3 (≥422 µg/L)	
	HR (95% CI)	P Value	HR (95% CI)	P Value
1†	0.87 (0.55-1.37)	.54	2.23 (1.48-3.34)	<.001
2‡	0.87 (0.52-1.46)	.60	1.91 (1.15-3.18)	.01
3§	0.86 (0.51-1.45)	.57	1.97 (1.16-3.33)	.01
4	0.89 (0.52-1.52)	.68	1.93 (1.14-3.27)	.02

Abbreviations: CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol.

*Lowest tertile (<310 µg/L) is the referent.

†Adjusted for age, sex, and race.

‡Adjusted for age, sex, race, smoking status, systolic blood pressure, LDL-C and HDL-C level, and diabetes.

§Adjusted for age, sex, race, smoking status, systolic blood pressure, LDL-C and HDL-C levels, diabetes, and hs-CRP level.

||Adjusted for age, sex, race, smoking status, systolic blood pressure, LDL-C and HDL-C levels, diabetes, hs-CRP level, antihypertensive medication, and body mass index.

COMMENT

In the ARIC population, levels of Lp-PLA₂ and CRP were higher in middle-aged Americans who subsequently had an ischemic stroke than in those who did not. As previously reported,²⁴ traditional risk factors such as diabetes, hypertension, age, and race were significantly associated with stroke in ARIC. Levels of LDL-C did not differ between incident stroke cases and noncases, and in fully adjusted models, LDL-C, HDL-C, and triglyceride levels were not associated with increased risk for stroke, as reported previously.⁵

One small study of 33 patients identified at least 2 months after an ischemic stroke found that the mean ± SD activity of Lp-PLA₂ was significantly higher in cases than in healthy controls (41 ± 18 nmol/mL per minute vs 29 ± 17 nmol/mL per minute).²⁵ While the present report was in preparation, another case-cohort study, performed in Rotterdam and including 110 ischemic stroke cases and a random sample of 1820 subjects, reported that the HR for stroke in the highest quartile for Lp-PLA₂ activity compared with the lowest quartile was 1.97 after adjustment for age, sex, and known cardiovascular risk factors.²⁶ In the present study, we measured Lp-PLA₂ mass by enzyme-linked immunosorbent assay, which in general has a high correlation with Lp-PLA₂ activity.²⁷ A limitation of this study is that levels of CRP and Lp-PLA₂ were based on 1 measurement at a single time point, whereas clinical recommendations for CRP evaluation recommend 2 measurements.¹⁹

Although lipid fractions such as those of LDL-C and HDL-C were strongly associated with development of CHD events in ARIC,³ LDL-C level did not differ significantly between cases and controls in the present study (136.6 vs 132.0 mg/dL [3.53 vs 3.41 mmol/L]), and in the model containing traditional risk factors, none of the lipid fractions were associated with incident stroke as previously

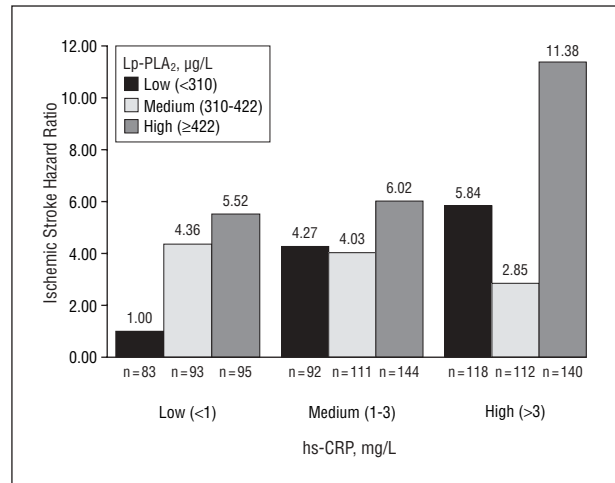


Figure. Association of lipoprotein-associated phospholipase (Lp-PLA₂) and high-sensitivity C-reactive protein (hs-CRP) with incident ischemic stroke. Ischemic stroke risk in individuals with elevated levels of both Lp-PLA₂ (highest tertile) and hs-CRP (high-risk category as defined in the Centers for Disease Control and Prevention/American Heart Association guidelines¹⁹) was more than 11 times greater than in individuals with low levels (first tertile) of Lp-PLA₂ and hs-CRP ($P < .001$; 95% confidence interval, 3.13-41.41). Compared with the individuals in the lowest categories of both variables, individuals in each of the other categories had significantly increased stroke risk except for individuals in the highest category of CRP and the middle tertile of Lp-PLA₂.

reported.⁵ In the Cardiovascular Health Study,²⁸ which enrolled Americans older than 65 years in 4 different geographic locations and also included a substantial number of African Americans, levels of LDL-C did not differ significantly between incident stroke cases and subjects who did not develop a stroke (132 vs 130 mg/dL [3.41 vs 3.36 mmol/L]). A large meta-analysis has shown that there is no association between total cholesterol level and stroke except in those screened younger than 45 years.²⁹

In contrast to lipid levels, levels of CRP and Lp-PLA₂ were associated with both incident CHD²⁰ and ischemic stroke. The differences observed in the ARIC population in the association of lipids with incident CHD vs incident stroke may reflect important differences in the pathophysiologic characteristics of these disorders. Coronary heart disease is usually due to atherosclerosis, the key pathologic features of which include intimal proliferation and lipid deposition. In contrast, ischemic strokes are due to a variety of disorders, including emboli from the heart or aorta, carotid atherosclerosis, and intracerebral vascular disease of small arteries, which in general presents a different pathologic finding than atherosclerosis of coronary arteries. In ARIC, more than one quarter of ischemic strokes were classified as lacunar strokes on the basis of size and anatomic location.⁵ Histopathologic studies performed decades ago showed that the most common pathologic finding in the smaller arteries of the brain after stroke were adventitial proliferation and vascular fibrosis, whereas intimal changes or lipid deposition were rarely observed.³⁰ Lipoprotein-associated phospholipase A₂, which is bound primarily to small, dense LDL, is responsible for the hydrolysis of oxidized phospholipids and the generation of lysophosphatidylcholine, which can lead to increased expression of adhesion molecules³¹ and thus could promote inflammation. High levels of CRP may also up-regulate adhesion mol-

ecule and chemokine expression to promote vascular inflammation.^{32,33} Therefore, Lp-PLA₂ and CRP, along with factors that promote endothelial dysfunction and inflammation such as hypertension, diabetes, and tobacco, may drive both intimal proliferation in large arteries, which are susceptible to atherosclerosis, and adventitial proliferation and fibrosis in smaller cerebral arteries.

If LDL-C is not a major risk factor for stroke, then how can one explain the reduction of stroke observed in statin trials? The recently published results on stroke reduction with simvastatin in the Heart Protection Study³⁴ show no association in the placebo group between incidence of stroke and baseline LDL-C level (5.7% stroke incidence for LDL-C <116 mg/dL [<3.0 mmol/L]; 5.4% for LDL-C ≥ 135 mg/dL [≥ 3.5 mmol/L]) or baseline level of HDL-C (5.6% stroke incidence for HDL-C <35 mg/dL [<0.9 mmol/L]; 5.7% for HDL-C ≥ 43 mg/dL [≥ 1.1 mmol/L]). In contrast, CHD event rates in placebo patients were associated with baseline LDL-C level (22.2% for LDL-C <116 mg/dL [<3.0 mmol/L]; 27.2% for LDL-C ≥ 135 mg/dL [≥ 3.5 mmol/L]) and HDL-C level (29.9% for HDL-C <35 mg/dL [<0.9 mmol/L]; 20.9% for HDL-C ≥ 43 mg/dL [≥ 1.1 mmol/L]).³⁵ Nonetheless, simvastatin therapy was associated with an approximate 25% reduction in stroke and CHD events in all categories of lipids. Although statins have a pronounced effect on lowering levels of LDL-C, statins significantly reduce the levels of CRP (by 20%-40%)³⁶ and also significantly reduce Lp-PLA₂ activity (by 28%-42%),³⁷ reductions similar to the event reduction observed in stroke.

Alternatively, the reductions in CRP and Lp-PLA₂ levels observed with statin therapy may not be mechanistically linked to reductions in stroke or myocardial infarction but may be markers of other anti-inflammatory effects of statins.³⁸ Fibrates, which are peroxisome proliferator-activated α -receptor agonists, have been shown to reduce levels of CRP and Lp-PLA₂.^{39,40} In the Veterans Affairs HDL Intervention Trial,⁴¹ the fibrate gemfibrozil reduced incident stroke without any reduction in level of LDL-C. If reductions in Lp-PLA₂ level with fibrates and statins are related to the reduction in stroke observed in clinical trials with these agents, then Lp-PLA₂ may be a novel target for therapy to reduce stroke risk; an agent that inhibits Lp-PLA₂ is currently in phase 3 development.⁴²

In summary, Lp-PLA₂ and CRP levels may be complementary to traditional risk factors to identify middle-aged individuals at increased risk for stroke. Future studies should determine whether selective inhibition of Lp-PLA₂ or reduction and/or inhibition of CRP reduces ischemic stroke and whether statins and/or fibrates are more effective for stroke prevention in patients with elevated levels of Lp-PLA₂.

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Author Affiliations: Section of Atherosclerosis and Lipoprotein Research, Department of Medicine, Baylor College of Medicine, and Center for Cardiovascular Disease Prevention, Methodist DeBakey Heart Center, Houston, Tex (Drs Ballantyne and Hoogeveen); Department of Biostatistics, School of Public Health, The University of North Carolina at Chapel Hill (Drs Bang and Chambless); Department of Epidemiology, Johns Hopkins Bloomberg

School of Public Health, Johns Hopkins University, Baltimore, Md (Drs Coresh and Sharrett); Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis (Dr Folsom); National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Md (Dr Myerson); and Vascular Biology Research Center, Department of Internal Medicine (Dr Wu), and the Human Genetics Center, School of Public Health (Dr Boerwinkle), The University of Texas Health Science Center at Houston.

Correspondence: Christie M. Ballantyne, MD, Baylor College of Medicine, 6565 Fannin, Mail Stop A-601, Houston, TX 77030 (cmb@bcm.tmc.edu).

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