Lipoprotein-Associated Phospholipase A_2, Hormone Use, and the Risk of Ischemic Stroke in Postmenopausal Women

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Abstract—Few studies have investigated the role of elevated lipoprotein-associated phospholipase A2 (Lp-PLA_2) with stroke risk, and those that have are based on small numbers of strokes. No study has evaluated the effect of hormone therapy use on the association of Lp-PLA_2 and stroke. We assessed the relationship between Lp-PLA_2 and the risk of incident ischemic stroke in 929 stroke patients and 935 control subjects in the Hormones and Biomarkers Predicting Stroke Study, a nested case-control study from the Women's Health Initiative Observational Study. Mean (SD) levels of Lp-PLA_2 were significantly higher among case subjects (309.0 [97.1]) than control subjects (296.3 [87.3]; $P<0.01$). Odds ratio for ischemic stroke for the highest quartile of Lp-PLA_2, compared with lowest, controlling for multiple covariates, was 1.08 (95% CI: 0.75 to 1.55). However, among 1137 nonusers of hormone therapy at baseline, the corresponding odds ratio was 1.55 (95% CI: 1.05 to 2.28),whereas there was no significant association among 737 hormone users (odds ratio: 0.70; 95% CI: 0.42 to 1.17; *P* for interaction=0.055). Moreover, among nonhormone users, women with high C-reactive protein and high Lp-PLA2 had more than twice the risk of stroke (odds ratio: 2.26; 95% CI: 1.55 to 3.35) compared with women low levels in both biomarkers. Furthermore, different stroke cases were identified as high risk by Lp-PLA_2 rather than by C-reactive protein. Lp-PLA_2 was associated with incident ischemic stroke independently of C-reactive protein and traditional cardiovascular risk factors among nonusers of hormone therapy with highest risk in those who had both high C-reactive protein and high Lp-PLA_2. **(*Hypertension*. 2008;51:1115-1122.)**

Key Words: stroke ■ lipoprotein-associated phospholipase A_2 ■ Lp-PLA_2 ■ postmenopausal women ■ stroke biomarkers ■ hormones ■ Women's Health Initiative ■ WHI

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an enzyme secreted by macrophages, largely bound to low-density lipoprotein cholesterol (LDL-C) in circulation. There is increasing interest in the proinflammatory properties of Lp-PLA2,[1,2] which is an important component of the cascade of events that involves the oxidation of LDL-C and may lead to plaque rupture. Lp-PLA2 has been shown to be expressed more in the necrotic core of advanced atherosclerotic plaque compared with less advanced lesions[3]; thus, it may be a marker or mediator of unstable plaque. Elevated circulating levels of Lp-PLA_2 have been shown to be independent predictors of coronary heart disease (CHD) in the Atherosclerosis Risk in Communities Study,[4] West of Scotland Coronary Prevention Study,[5] and the Rotterdam Study,[6] as well as in some others.[7–10]

Studies relating stroke risk to Lp-PLA_2 have generally involved modest numbers of strokes. The Atherosclerosis Risk in Communities Study included 194 stroke cases and found highest risk in individuals with both high C-reactive protein (CRP) and high Lp-PLA_2 levels.[4] The Rotterdam Study, which included 110 stroke subjects, found a 2-fold increase in risk of ischemic stroke among persons in the highest quartile of Lp-PLA_2 levels compared o the lowest quartile, after adjustment. The Women's Health Study,[11] which examined the relationship of Lp-PLA_2 with combined CHD and stroke end points in 123 case subjects and 123 control subjects, found no significant relationship. However, there have been no large or long-term studies of the relationship of Lp-PLA_2 and ischemic stroke in older women, nor any studies exploring the interaction between hormone therapy (HT) and the predictive strength of Lp-PLA_2. Because Lp-PLA_2 has been shown to be lower among hormone users,[11] it was decided a priori to test for an interaction between Lp-PLA_2 and HT in this group of postmenopausal women.

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This report presents data from the Hormones and Biomarkers Predicting Stroke (HaBPS) Study, a case-control study of 972 stroke subjects and their matched control subjects nested in the Women's Health Initiative (WHI) Observational Study. We addressed the question of whether Lp-PLA_2 is a predictor of stroke independent of traditional risk factors and whether HT modifies the association between Lp-PLA_2 and stroke.

Materials and Methods

Design and Study Population

The HaBPS Study is a case-control study of 972 incident ischemic stroke subjects and 972 matched control subjects nested within the WHI Observational Study. Briefly, the WHI, conducted in 40 clinical centers across the United States, was designed to examine the impact of a number of factors on many of the major causes of morbidity and mortality in postmenopausal women. Eligible women were 50 to 79 years of age at baseline, postmenopausal, had no medical conditions associated with a predicted survival of <3 years, and provided informed consent to be a part of the study, as approved by the institutional review boards. Enrollment took place from October 1993 to December 1998. The WHI methods are described in detail elsewhere.[12,13] Stroke case and control subjects came from the 93 676 women enrolled in WHI Observational Study, with mean±SD follow-up in control subjects of 7.9±1.3 years and range from 1.9 to 10.5 years.

Of 93 676 participants in the WHI Observational Study, 10 458 were excluded from the HaBPS Study: 9831 did not meet eligibility criteria of the HaBPS Study, which were no previous history of myocardial infarction or stroke and adequate blood sample available for the biomarker assays; and 627 participants with a locally adjudicated ischemic stroke, which was not confirmed as ischemic stroke by central adjudication were also excluded as control or case subjects. The remaining cohort consisted of 82 591 women from among whom the stroke case and control subjects were selected. There were 972 ischemic subjects and 972 control subjects. Control selection was done in a time-forward manner, selecting 1 control for each case from the risk set at the time of the case subject's event. Matching was performed on age at screening (±2 years), race/ethnicity (white, black, Hispanic, Asian, American Indian, or other/unspecified), date of study enrollment (±3 months), and follow-up time (control follow-up time ≥ case follow-up time). Race/ethnicity was matched exactly, and the continuous matching variables were selected based on a criterion to minimize an overall distance measure. Case and control subjects were pulled from separate data sets, so case subjects could not be selected as control subjects.

Data Collection

All of the women enrolled in WHI completed baseline visits to determine eligibility and to collect data including questionnaires, physical examinations, biological specimens, and laboratory tests. A physical examination was performed by certified staff using standardized procedures to obtain height and weight and blood pressure. Diastolic and systolic blood pressure were measured in the right arm with a conventional mercury sphygmomanometer after the participant was seated and rested for 5 minutes. The cuff of appropriate size was determined by arm circumference. SBP was defined as the pressure level at which the first ≥2 knocking sounds occurred in appropriate rhythm. Diastolic blood pressure was phase V Korotkoff value (disappearance of sound). The averages of 2 measurements taken 30 seconds apart were used in analyses. Hypertension was defined as either self-reported current medication use or elevated clinic blood pressure (SBP ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg). Diabetes was defined as self-reported treatment or a fasting glucose level ≥126 mg/dL. Metabolic syndrome was based on National Cholesterol Education Program Adult Treatment Panel III criteria.[14]

Women were queried about lifestyle factors, medical history, and personal habits and were asked to bring all of their prescription medications in their original bottles to the baseline visit to be entered into a pharmacy database, the Master Drug Data Base (Medi-Span). Fasting blood samples were collected after a standardized protocol and labeled, centrifuged, and frozen on site in −70°C freezers and later shipped to the central WHI specimen repository (McKesson BioServices) for long-term storage.

Laboratory Analyses

The case and control samples were extracted from the specimen archive and sent from the biorepository to the laboratory at diaDexus for assay of Lp-PLA_2. Lp-PLA_2 mass was measured in plasma aliquots that were collected at the time of enrollment and stored at −70°C using an enzyme-linked immunoassay (PLAC test, diaDexus, Inc). Samples were incubated in microtiter plate wells with immobilized monoclonal antibody (2C10) against Lp-PLA_2. The enzyme was identified by a second monoclonal anti-Lp-PLA_2 antibody (4B4) labeled with horseradish peroxidase. The standard was recombinant Lp-PLA_2. The range of detection was 50 to 1000 ng/mL, and the interassay coefficients of variation were 7.8% at 276 ng/mL, 6.1% at 257 ng/mL, and 13.5% at 105 ng/mL. There was no cross-reactivity with other A2 phospholipases. All of the analyses were performed blinded to risk factors, and biochemical and clinical characteristics. Lipids were assayed at Liposcience using nuclear magnetic resonance. Inflammatory factors were assayed at Medical Research Laboratories. LDL-C was calculated from triglycerides, as well as high-density lipoprotein cholesterol (HDL-C) and total cholesterol for those women who had a triglyceride value <400. LDL-C values were set to missing for those women whose triglyceride value was >400 (n=35) or who were missing HDL-C, total cholesterol, or triglyceride values (n=7).

Follow-Up and Outcome Ascertainment

Study subjects were followed annually by mail or telephone to determine outcomes and overnight hospitalizations. Reports from participants or third-party sources also triggered further investigation. Details of illnesses and hospitalizations were obtained from medical charts. Local trained physicians adjudicated potential outcomes and assigned a diagnosis according to standard criteria. All of the locally adjudicated strokes were sent for central adjudication by trained neurologists. Locally adjudicated cases not verified during central adjudication as being a stroke were excluded. Ischemic stroke was defined as the rapid onset of a persistent neurologic deficit attributed to an obstruction lasting >24 hours without evidence for other causes, unless death supervened or there was a demonstrable lesion compatible with acute stroke on computed tomography or MRI scan. Only stroke events that required hospitalization were considered as a potential outcome. Strokes were classified as ischemic or hemorrhagic on review of reports of brain imaging studies. Transient ischemic attacks or hemorrhagic strokes were not included in the definition of stroke outcome.

Statistical Analyses

Univariate analyses were conducted to evaluate differences in covariates between matched case and control subjects using McNemar's χ^2 test. Spearman correlations between Lp-PLA_2 and other biomarkers, as well as standard stroke risk factors, were calculated among control subjects to assess for multicollinearity. Because Lp-PLA_2 was normally distributed in this sample of women, no transformations were necessary; however, log transformation was used for CRP. Paired *t* tests were used to compare mean levels of Lp-PLA_2 between matched case-control pairs. The risk of incident ischemic stroke was examined by using conditional logistic regression, which accounts for matching to estimate odds ratios (ORs) and 95% CIs for quartiles of Lp-PLA_2, with category cutpoints defined according to the distribution of controls (233.7, 285.0, and 352.6 ng/mL). We report *P* values for linear trends by modeling Lp-PLA_2 as a continuous variable. Multivariate models were additionally adjusted for cardiovascular risk factors that differed significantly between case and control subjects on mean Lp-PLA_2 and are known to be related to cardiovascular outcomes (aspirin use, body mass

index [BMI], diabetes, SBP, smoking, use of antihypertensive medication, LDL-C, and HDL-C). Models were further adjusted for hormone use, fasting glucose, waist circumference, LDL-C particle number, and CRP. Adjusted models were based on case-control pairs for whom complete data were available on all of the covariates of interest. Interactions among Lp-PLA$_2$ and age, HT, CRP (categorized according to clinical criteria as <1.0, 1.0 to 3.0, and >3.0), LDL-C, HDL-C, systolic blood pressure, hypertension, Framingham risk score, and metabolic syndrome were explored by running logistic regression models, which included Lp-PLA$_2$, the variable of interest, and the interaction term, separately for each of the variables listed above, using the likelihood ratio test.

Investigation of the effect of HT on the association of Lp-PLA$_2$ and stroke was prespecified, and, accordingly, analyses were done separately among hormone users and nonusers. Stratified analyses were conducted using unconditional logistic regression on all of the available control and case subjects having Lp-PLA$_2$ assays. Because case-control pairs are not concordant on the stratification variables, matching could not be maintained within strata. SAS 9.1 was used for all of the analyses (SAS Institute Inc).

Results

Not all of the 972 matched pairs had adequate specimens to assay LpPla2; there were 935 control subjects and 929 case subjects who did have adequate samples for Lp-PLA$_2$ assays. Baseline characteristics of all of the case and control subjects with Lp-PLA$_2$ assays are shown in Table 1. Case subjects were more likely to be current smokers; have higher BMI; report a history of atrial fibrillation, angina, or revascularization; have hypertension, diabetes, and/or lower HDL-C levels; and use aspirin. Case subjects had significantly higher mean levels of Lp-PLA$_2$ (309.0 ng/mL, SD=97.1) than control subjects (296.3 ng/mL, SD=87.3; $P \leq 0.01$). Table 1 shows mean levels of Lp-PLA$_2$ in case and control subjects by baseline characteristics. Lower mean levels of Lp-PLA$_2$ among control subjects are found in black women, hormone users, diabetic subjects, in aspirin users, and in women with lower levels of LDL-C and higher levels of CRP and HDL-C. Correlations of Lp-PLA$_2$with lipids accounted for only a small proportion of the variance (LDL-C: $r=0.38$; total cholesterol: $r=0.30$; HDL-C: $r=-0.20$; data not shown). The correlations of Lp-PLA$_2$ with standard risk factors were also low. The highest relationships were inverse ones with hormone use ($r=-0.19$; $P<0.0001$) and aspirin use ($r=-0.11$; $P<0.001$).

Among the 935 control subjects and 929 case subjects with Lp-PLA$_2$, there were 895 pairs with measurements of Lp-PLA$_2$ in both case subjects and their matched control subjects. Conditional logistic regression analysis of these 895 matched case-control pairs indicates that, in unadjusted models, there is a significant trend for increasing ORs with higher quartiles of Lp-PLA$_2$ ($P<0.01$), with the OR for ischemic stroke being 39% higher in the highest quartile compared with the lowest quartile (OR: 1.39; 95% CI: 1.04 to 1.86; Table 2). This association was no longer significant with adjustment for traditional cardiovascular risk factors (OR: 1.08; 95% CI: 0.75 to 1.55; P for trend=0.36). In addition, controlling for hormone use, glucose, waist circumference, LDL-C particle number, or CRP did not alter these findings for the whole group of women. Unconditional analyses of the 935 control subjects and 929 stroke case subjects gave essentially the same results.

In models adjusted for cardiovascular risk factors, SBP was significantly associated with case status (OR per 10 mm Hg: 1.21; 95% CI: 1.14 to 1.29 mm Hg), as was use of any antihypertensive medication at baseline (OR: 1.39; 95% CI: 1.11 to 1.72). Likelihood ratio tests indicated no significant interactions between Lp-PLA$_2$ and SBP or hypertension or between Lp-PLA$_2$ with age, CRP, LDL-C, HDL-C, metabolic syndrome, or Framingham risk score (data not shown), but the interaction between current HT use and Lp-PLA$_2$ was suggestive, with $P=0.055$. Because effect modification by HT use was of a priori interest, Lp-PLA$_2$ quartile analyses done separately among the nonusers and users of HT at WHI baseline are shown in Table 2. Among non-HT users, after adjusting for CVD risk factors, there was a 55% higher risk of stroke for those in the highest compared with the lowest quartile of Lp-PLA$_2$ (OR: 1.55; 95% CI: 1.05 to 2.28; P for trend<0.01). Additional adjustment for glucose, waist circumference, LDL-C particle number, or CRP did not alter these results. Among users of HT there was no significant relationship between Lp-PLA$_2$ and risk of stroke. To assess whether HT use also modified the CRP relationship with stroke, we ran a parallel analysis of CRP effects in users and nonusers of HT (Table 3). The association of CRP and stroke, comparing CRP >3.0 with CRP <1.0, was also stronger among nonusers of HT (OR: 1.65; 95% CI: 1.15 to 2.36) than among users (OR: 1.10; 95% CI: 0.63 to 1.93); although the CIs overlap.

We conducted stratified analyses within the group of 1137 nonhormone users at WHI baseline, comparing those with Lp-PLA$_2$ levels above the mean of control subjects (296.3 ng/mL) with those below the mean. Among nonusers, the interaction with Framingham risk score had a P value of 0.05; significant effects of Lp-PLA$_2$ were seen in the highest tertile of Framingham risk (OR: 2.07; 95% CI: 1.38 to 3.11), and no significant effect of Lp-PLA$_2$ was seen in the lower tertiles of Framingham risk. A similar pattern was found by tertiles of CRP (P for interaction=0.07). In the fully adjusted model, the OR associated with being above the mean of Lp-PLA$_2$ versus below the mean among those whose CRP was >3.0 was 69% higher (adjusted OR: 1.69; 95% CI: 1.11 to 2.56). We further examined the interactions by looking at the effect of Lp-PLA$_2$ in combination with low or high levels of CRP (Figure). CRP was dichotomized as below or above 3.0 mg/dL, and Lp-PLA$_2$ was dichotomized based on the mean of control subjects. In nonusers of HT, those women with high CRP and high Lp-PLA$_2$ compared with those with low CRP and low Lp-PLA$_2$ had 2.26 times the risk of stroke after adjustment for cardiovascular risk factors (OR: 2.26; 95% CI: 1.52 to 3.35).

To investigate whether Lp-PLA$_2$ and CRP may identify different cases of stroke, we examined the 483 strokes (51% of stroke cases) that were discordant on risk predicted by these 2 biomarkers at baseline. We compared the baseline characteristics of the 225 case subjects suggested to be at high risk because of elevated baseline levels of Lp-PLA$_2$ but not having elevated CRP, with the 258 case subjects having elevated CRP at baseline but not elevated Lp-PLA$_2$. Those identified as high risk by high Lp-PLA$_2$ (but not by CRP) compared with those identified by high CRP (but not Lp-

Table 1. Baseline Characteristic and Mean Lp-PLA$_2$ Levels by Case-Control Status

	Control Subjects (n=935)		Case Subjects (n=929)	
Variable	n (%)	Lp-PLA$_2$ Mean (SD)	n (%)	Lp-PLA$_2$ Mean (SD)
Demographics				
Age groups, y				
50 to 59	93 (10.0)	286.0 (97.5)	92 (9.9)	298.8 (89.6)
60 to 69	376 (40.2)	291.2 (86.2)	374 (40.3)	302.8 (94.3)
70 to 79	466 (49.8)	302.4 (85.8)	463 (49.8)	316.1 (100.3)
Race/ethnicity				
American Indian/Alaskan Native	5 (0.5)	318.2 (102.6)*	5 (0.5)	351.4 (65.1)†
Asian/Pacific Islander	21 (2.3)	244.1 (72.1)	21 (2.3)	297.3 (105.7)
African American	78 (8.3)	269.1 (96.0)	74 (8.0)	276.8 (90.8)
Hispanic	20 (2.1)	303.0 (75.5)	20 (2.2)	278.4 (58.2)
Other	13 (1.4)	266.6 (71.1)	12 (1.3)	298.6 (100.9)
White	798 (85.4)	300.5 (86.4)	797 (85.8)	313.0 (97.8)
Risk factors				
Smoking				
Never	506 (54.6)	298.4 (86.0)	482 (52.5)	308.6 (93.3)
Past	385 (41.6)	292.0 (89.0)	363 (39.5)	305.7 (104.4)
Current	35 (3.8)	313.5 (87.5)	73 (8.0)	322.6 (82.1)
Alcohol				
Nondrinker	108 (11.6)	299.9 (81.0)	112 (12.1)	309.0 (89.1)
Past drinker	170 (18.2)	299.3 (97.6)	200 (21.6)	301.0 (91.6)
<1 drink per month	117 (12.5)	295.1 (74.9)	108 (11.7)	323.3 (112.5)
<1 drink per week	197 (21.1)	304.9 (93.5)	197 (21.3)	315.7 (103.2)
1 to <7 per week	228 (24.4)	288.1 (81.7)	192 (20.7)	302.5 (94.2)
≥7 per week	113 (12.1)	290.1 (88.3)	117 (12.6)	309.2 (91.8)
Body mass index				
<25	376 (40.8)	294.2 (85.6)	319 (34.7)	306.5 (87.6)
25 to 30	332 (36.1)	299.3 (91.1)	348 (37.8)	306.4 (94.4)
>30	213 (23.1)	296.6 (84.9)	253 (27.5)	316.7 (112.0)
Hormone use				
No current hormone use	577 (61.7)	307.9 (84.6)*	560 (60.3)	327.2 (97.8)†
Any current hormone use	358 (38.3)	277.4 (88.5)	369 (39.7)	281.4 (89.2)
Type of hormone use				
Estrogen alone	229 (64.0)	274.8 (93.4)*	263 (71.3)	273.7 (76.8)†
Estrogen+progestin	129 (36.0)	282.2 (79.2)	106 (28.7)	300.6 (112.5)
Diastolic blood pressure				
<90	888 (95.0)	296.1 (87.2)	853 (92.2)	309.3 (97.2)
≥90	47 (5.0)	298.5 (90.9)	72 (7.8)	305.5 (93.8)
SBP				
≤120	314 (33.6)	292.6 (88.2)	188 (20.3)	299.1 (83.1)
120 to 140	371 (39.7)	295.4 (82.2)	358 (38.6)	307.4 (83.6)
>140	250 (26.7)	302.0 (93.5)	381 (41.1)	315.7 (113.6)
Hypertension				
No	513 (55.4)	295.8 (85.2)	334 (36.3)	304.7 (80.4)
Yes	413 (44.6)	295.7 (89.2)	585 (63.7)	310.4 (105.3)
Diabetes				
No	854 (91.5)	298.3 (87.2)*	774 (83.5)	308.8 (95.9)
Yes	79 (8.5)	273.8 (86.7)	153 (16.5)	311.7 (103.0)

(*Continued*)

Table 1. Continued

	Control Subjects (n=935)		Case Subjects (n=929)	
Variable	n (%)	Lp-PLA$_2$ Mean (SD)	n (%)	Lp-PLA$_2$ Mean (SD)
Metabolic syndrome				
No	619 (66.3)	291.8 (83.8)*	481 (52.1)	300.0 (86.2)†
Yes	315 (33.7)	305.3 (93.3)	443 (47.9)	319.6 (106.8)
Framingham score				
Tertile 1	300 (33.0)	292.6 (84.8)	194 (21.7)	293.4 (84.0)†
Tertile 2	302 (33.3)	300.7 (86.7)	275 (30.7)	308.1 (96.8)
Tertile 3	306 (33.7)	296.0 (89.3)	426 (47.6)	314.4 (101.6)
Comorbidities				
History of atrial fibrillation				
No	863 (94.4)	297.0 (86.4)	819 (90.1)	307.2 (96.2)
Yes	51 (5.6)	285.4 (95.1)	90 (9.9)	319.1 (102.7)
History of angina				
No	871 (94.4)	296.8 (87.8)	837 (90.7)	308.3 (97.8)
Yes	52 (5.6)	282.2 (71.8)	86 (9.3)	314.1 (92.0)
History of revascularization				
No	902 (98.8)	296.1 (87.0)	873 (96.0)	307.7 (97.2)
Yes	11 (1.2)	301.4 (74.4)	36 (4.0)	325.5 (87.7)
Medications				
Use of any hypertensive medication				
No	609 (65.1)	299.2 (87.0)	493 (53.1)	305.7 (85.7)
Yes	326 (34.9)	290.7 (87.8)	436 (46.9)	312.8 (108.5)
Aspirin use				
No	702 (75.1)	301.7 (89.1)*	646 (69.5)	308.2 (94.2)
Yes	233 (24.9)	279.8 (79.7)	283 (30.5)	311.0 (103.4)
High cholesterol requiring pills				
No	778 (84.8)	294.4 (86.7)	737 (81.4)	305.4 (94.5)
Yes	139 (15.2)	300.3 (89.0)	169 (8.7)	319.8 (108.5)
Biomarkers				
CRP				
<1.0	195 (21.5)	300.9 (85.2)*	126 (14.0)	307.9 (90.2)†
1.0 to 3.0	319 (35.1)	306.9 (86.7)	277 (30.7)	323.3 (108.8)
>3.0	395 (43.5)	286.1 (89.3)	499 (55.3)	300.7 (91.5)
LDL-C				
<100	142 (15.4)	243.6 (65.0)*	114 (12.6)	250.3 (73.3)†
100 to 130	243 (26.4)	276.9 (73.0)	239 (26.4)	282.3 (73.4)
≥130	537 (58.2)	319.0 (90.6)	553 (61.0)	332.7 (101.3)
HDL-C				
<40	79 (8.5)	320.7 (99.7)*	110 (11.9)	360.1 (138.7)†
40 to 60	426 (45.6)	306.4 (87.9)	466 (50.3)	312.8 (92.2)
≥60	430 (46.0)	281.7 (82.0)	351 (37.9)	288.0 (80.1)

*$P<0.05$ for the difference in mean Lp-PLA2 levels among controls for baseline variables.
†$P<0.05$ for the difference in mean Lp-PLA2 levels among cases for baseline variables.

PLA$_2$) were older, had lower BMI, higher levels of LDL-C, and were nonhormone users. Adjusting for these significant baseline differences, we found that nonhormone users were 6 times more likely to be identified by Lp-PLA$_2$ than by CRP (OR: 5.78; 95% CI: 3.58 to 9.31; Table 4).

Discussion

We found, in the largest study of ischemic strokes of older women to date, that among women not using HT, levels of the biomarker Lp-PLA$_2$ in the highest quartile compared with those in the lowest are independently associated with a 64%

Table 2. Risk of Ischemic Stroke by Quartile of Lp-PLA$_2$

	Quartile Categories of Lp-PLA$_2$				
Variable	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for Trend
All women					
Unadjusted (895 matched pairs)					
OR	1	1.01	1.23	1.39	0.01
95% CI	(Reference)	(0.76 to 1.33)	(0.93 to 1.63)	(1.04 to 1.86)	
Adjusted for CVD risk factors (817 matched pairs)*					
OR	1	0.86	1.16	1.08	0.36
95% CI	(Reference)	(0.63 to 1.18)	(0.85 to 1.60)	(0.75 to 1.55)	
Adjusted for CVD risk factors and hormone use (817 matched pairs)					
OR	1	0.87	1.19	1.12	0.28
95% CI	(Reference)	(0.63 to 1.20)	(0.86 to 1.65)	(0.77 to 1.62)	
Nonusers of HT					
Adjusted for matching factors (560 cases, 577 controls)†					
OR	1	0.97	1.22	1.64	<0.01
95% CI	(Reference)	(0.67 to 1.42)	(0.85 to 1.74)	(1.16 to 2.33)	
Adjusted for matching factors and CVD risk factors (541 cases and 552 controls)					
OR	1	0.92	1.26	1.55	<0.01
95% CI	(Reference)	(0.62 to 1.38)	(0.86 to 1.84)	(1.05 to 2.28)	
Users of HT					
Adjusted for matching factors (369 cases and 358 controls)					
OR	1	1.00	1.26	0.95	0.81
95% CI	(Reference)	(0.68 to 1.46)	(0.84 to 1.89)	(0.62 to 1.46)	
Adjusted for matching factors and CVD risk factors (344 cases and 346 controls)					
OR	1	0.91	1.15	0.70	0.43
95% CI	(Reference)	(0.60 to 1.38)	(0.73 to 1.81)	(0.42 to 1.17)	

*CVD risk factors: aspirin use, body mass index, diabetes, SBP, smoking, hypertensive medication use, LDL-C, and HDL-C.
†Matching factors: age and race.

increased risk of ischemic stroke. The increased risk persists after adjustment for traditional cardiovascular risk factors (OR: 1.55; 95% CI: 1.05 to 2.28) and is unchanged with additional adjustment for CRP (OR: 1.48; 95% CI: 1.00 to 2.19). There was no association of Lp-PLA$_2$ with stroke risk among women using HT. At the time of the baseline visit in our study, 61% of the women were nonusers of HT. The proportion of nonusers of HT may be even higher in later

Table 3. Adjusted ORs for Ischemic Stroke by Clinical Category of CRP

	Clinical Categories of CRP			
Variable	<1.0	1.0 to 3.0	>3.0	*P* for Trend*
Non-HT users (553 cases and 559 controls)				
OR	1	1.15	1.65	<0.01
95% CI	(Reference)	(0.82 to 1.61)	(1.15 to 2.36)	
HT users (343 cases and 348 controls)				
OR	1	1.06	1.10	0.04
95% CI	(Reference)	(0.59 to 1.89)	(0.63 to 1.93)	

ORs were adjusted for age, race, and CVD risk factors: aspirin use, body mass index, diabetes, systolic blood pressure, smoking, hypertensive medication use, LDL-C, and HDL-C.
**P* for trend is Wald χ^2 for log normal CRP entered as a continuous variable.

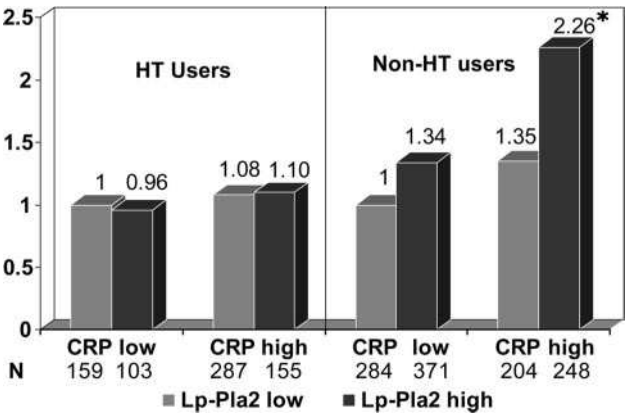

Figure. Adjusted ORs of stroke by CRP and Lp-PLA$_2$ high and low levels. Low Lp-PLA2 is ≤296.3 ng/mL. Reference group: low CRP (≤3.0 mg/d for age, race, and CVD risk factors). *$P<0.05$.

years of follow-up, because the results of the WHI clinical trial of HT, published in 2002 for the estrogen plus progestin trial,[15] and in 2004 for the estrogen alone trial,[16] indicated an excess risk of stroke[17,18] and other end points, including breast cancer. Thus, for postmenopausal women, it is important to examine biomarker effects in nonusers of HT. To our knowledge this is the first study to do so.

Among nonusers of hormones, the association of Lp-PLA$_2$ with stroke risk was strongest in women who also had elevated CRP, consistent with findings from the Atherosclerosis Risk in Communities Study.[19] The stroke risk in the highest quartile of Lp-PLA$_2$ compared with the lowest quartile was robust and consistent (between 1.5 and 1.6) in models that adjusted for multiple combinations of risk factors and independent of CRP. The correlation between Lp-PLA$_2$ and CRP among the control subjects was −0.10, accounting for <1% of the variance of CRP. The combined effect of high CRP and high Lp-PLA$_2$ was associated with twice the risk of stroke as compared with having low values of both biomarkers. Thus, high Lp-PLA$_2$ may have a similar predictive value as CRP in nonusers of HT and suggests higher risk when considered jointly with high levels of CRP. The reason for the robust association of Lp-PLA$_2$ and ischemic stroke in nonusers of HT and the absence of a significant relationship in users of HT requires further study. Because Lp-PLA$_2$ is higher in nonusers of HT, it may be that HT use blocks the associations of Lp-PLA$_2$ and stroke. Our data suggest that there may not be a high level of redundancy in risk stratification through CRP versus Lp-PLA$_2$, because different stroke cases were identified as high risk by 1 biomarker but not the other.

Table 4. Adjusted ORs for Stroke Cases Identified as High Risk by Lp-PLA$_2$ But Not by CRP (n=225) Compared With Stroke Cases Identified by CRP But Not Lp-PLA$_2$ (n=258)

Variable	OR	95% CI
Age, per 1-y increase	1.06	1.02 to 1.09
Race, nonwhite vs white	0.72	0.38 to 1.35
BMI, per 1 unit increase	0.93	0.89 to 0.98
Current hormone use, no vs yes	5.78	3.58 to 9.31
Diabetes, no vs yes	1.87	0.92 to 3.81
Any hypertension medication use, no vs yes	1.11	0.69 to 1.78
Metabolic syndrome, no vs yes	1.82	1.08 to 3.06
LDL-C, per 1 unit increase	1.02	1.01 to 1.03

Dependent variable is high Lp-PLA$_2$/low CRP (=1) vs low Lp-PLA$_2$/High CRP (=0). BMI indicates body mass index.

As expected, the risk of stroke increased with increasing systolic blood pressure adjusted for other cardiovascular risk factors for all of the women and for users and nonusers of HT separately. There was no interaction between hypertension or systolic blood pressure and Lp-PLA$_2$. The HaBPS Study has important strengths. Although other studies have found higher risks for CHD, as summarized in the meta-analysis by the Lp-PLA$_2$ Collaborative Group,[20] our study indicates that stroke is associated with high level of Lp-PLA$_2$, and it includes by far the largest number of ischemic strokes (n=929) studied to date with respect to Lp-PLA$_2$ and with long follow-up of an average of 8 years. Stroke was ascertained rigorously and centrally adjudicated. Most importantly, it examines the association of Lp-PLA$_2$ and ischemic stroke separately in the large group of nonusers of HT, which has not been investigated in other studies.

The results reported here must be viewed in the context of several limitations. The use of HT was by self-report; however, it is unlikely that there was differential self-reporting among those who did versus those who did not go on to develop stroke. Despite the fact that we controlled for numerous risk factors, including other blood biomarkers, which were measured at the same time as Lp-PLA$_2$, there may have been uncontrolled confounding. Power for comparisons among hormone users was lower than among nonusers, because there were more nonusers. We cannot estimate prospective stroke rates among those with high versus low levels of Lp-PLA$_2$, because this was a nested case-control study, and, thus, we cannot obtain hazard ratios based on time to event. The ORs that we obtained are a good estimate of relative risk if not absolute risk.

In conclusion, we found that Lp-PLA$_2$ was independently associated with incident ischemic stroke in postmenopausal women who were not using HT, which represents the majority of postmenopausal women. Highest risk was found in those with high levels of both Lp-PLA$_2$ and CRP. Different women were identified as high risk by each of these 2 biomarkers. The mechanisms underlying the association of Lp-PLA$_2$ and stroke in nonusers of HT and the absence of this association among the users needs to be explored further, especially in view of the decreased use of hormones since the publication of the WHI report that indicated that risks of hormone use outweighed their benefits.

Perspectives

This study has identified Lp-PLA$_2$ as an independent predictor of ischemic stroke among postmenopausal women not using HT. It identifies high-risk women who were not captured as high risk by CRP levels; however, at greatest risk are those with elevated values of both biomarkers. Further explorations on the interaction of Lp-PLA$_2$ and estrogen and progestin are warranted.

Appendix

The complete list of WHI centers and investigators can be found online at http://www.whiscience.org/publications/WHI_investigators_shortlist.pdf.

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Disclosures

None.

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