Published in final edited form as:
Thromb Res. 2013 July ; 132(1): 44–46. doi:10.1016/j.thromres.2013.05.014.

Lipoprotein-associated phospholipase A_2 and venous thromboembolism: a prospective study

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Abstract

Introduction—Plasma lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an inflammatory marker associated positively with atherothrombotic risk. Whether Lp-PLA_2 is related to risk of venous thromboembolism (VTE) is incompletely studied.

Methods—We assessed Lp-PLA_2 activity in 10,687 Atherosclerosis Risk in Communities (ARIC) Study participants and followed them a median of 8.3 years (from 1996–98 through 2005) for VTE occurrence ($n = 226$).

Results—There was no significant association between baseline Lp-PLA_2 quartiles and risk of VTE, neither overall nor stratified as provoked or unprovoked. Adjusted for other risk factors, the hazard ratios (95% confidence interval) of total VTE across quartiles of Lp-PLA_2 were 1.0 (reference), 0.95 (0.64, 1.42), 1.03 (0.69, 1.56), and 1.26 (0.83, 1.91). In the subset of participants with LDL-cholesterol ≥ 130 mg/dL, hazard ratios of total VTE were 1.00, 1.39 (0.44, 4.44), 2.45 (0.84, 7.11), and 2.84 (0.99, 8.14).

Conclusion—Our study does not support the overall hypothesis that elevated Lp-PLA_2 contributes to VTE occurrence in the general population. However, in the presence of high LDL-cholesterol there was some evidence that Lp-PLA_2 may increase VTE risk.

Keywords

lipoprotein-associated phospholipase A_2; prospective study; pulmonary embolism; venous thromboembolism

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Introduction

Plasma lipoprotein-associated phospholipase A_2 (Lp-PLA_2), also known as platelet activating factor acetylhydrolase, is produced by inflammatory cells, co-travels with low-density lipoprotein (LDL), and hydrolyzes oxidized phospholipids, thereby propagating inflammation and potentially thrombosis [1]. Prospective epidemiologic studies have consistently associated elevated Lp-PLA_2 (e.g., above the highest tertile) with increased risk of atherothrombotic events [2–5]. The hypothesized mechanism for Lp-PLA_2 elevating atherothrombotic risk is the generation of bioactive lipids that elicit an arterial inflammatory response [6].

Just one study, with only 129 incident elderly VTE cases, has examined whether elevated Lp-PLA_2 is associated with increased risk of VTE [7]. It found no relation of plasma Lp-PLA_2 activity or mass with VTE incidence. We hypothesized that a positive association exists between Lp-PLA_2 activity and VTE in a cohort of white and African American adults.

Methods

In 1987–89, the ARIC Study recruited to an initial examination a cohort of 15,792 men and women aged 45–64 years from four U.S. communities [8]. Participants were re-examined in 1990–92 (93% response), 1993–95 (86%) and 1996–98 (80%), and followed long-term for cardiovascular events. Participants in ARIC Visit 4 serve as the cohort for the present analysis.

ARIC assessed Lp-PLA_2 activity in Visit 4 plasma by an automated Colorimetric Activity Method (CAM) assay (diaDexus Inc., South San Francisco, CA) using a Beckman Coulter (Olympus) AU400e autoanalyzer. The Lp-PLA_2 activity assay had an inter-assay variation coefficient of 4.4% and a reliability coefficient (R) of 0.92, based on 419 blinded replicate samples. ARIC also measured cystatin C (N Latex Cystatin C, Dade Behring, Inc., Deerfield, IL) to estimate glomerular filtration rate (eGFR) [9], and assessed plasma C-reactive protein (CRP) by the CRP-Latex (II) high sensitivity assay from Denka Seiken (Tokyo, Japan). ARIC did not measure factor $VIII_c$ or activated partial thromboplastin time (aPTT) at Visit 4, so Visit 1 values were used [10,11].

ARIC followed participants through 2005 to identify hospitalized VTE events. Physician-reviewers validated VTEs using a standardized protocol involving review of medical records and imaging reports [12]. Reviewers also subcategorized VTEs as unprovoked or provoked (occurring within 90 days of major trauma, surgery, marked immobility, active cancer or chemotherapy) [12].

From the 11,656 participants at Visit 4, we excluded from analyses 342 who had a prior history of VTE, 191 who were taking warfarin, 31 non-white or non-African American participants, 403 with missing Lp-PLA_2 values, and two Lp-PLA_2 outliers. Our final analytic sample comprised 10,687 participants -- 8328 whites, 2359 African Americans. Follow-up time ended when the participant had a VTE, died, was lost to follow-up, or survived until 31 December 2005.

We used Cox proportional hazards regression to model the association between quartiles of baseline (Visit 4) Lp-PLA_2 and VTE incidence and to derive hazard ratios and 95% CI. We tested the trend in hazard ratios using an ordinal variable to designate each quartile. In a secondary analysis, we also examined the VTE association using Lp-PLA_2 as a continuous variable. The proportional hazards assumption was tested for total, provoked, and unprovoked VTE models by including time by Lp-PLA_2 interaction terms. The p-value for

this proportionality test ranged from 0.02 to 0.11, and analyses stratified by time suggested Lp-PLA_2 might be positively associated with VTE in the first five years of follow-up and negatively during later follow-up. However, in neither period was the p-trend for the association statistically significant. Therefore, we chose to present time-unstratified results. Covariates in the models included VTE risk factors measured in the whole ARIC cohort at Visit 4 [10,11,13,15] as well as LDL-cholesterol and statin use. Adjustment for factor $VIII_c$ and aPTT (measured at Visit 1) had no impact, so they were dropped from models. Smoking, physical activity, and hypertension were not VTE risk factors in ARIC [13].

Results

In this sample of 10,687 ARIC Visit 4 participants aged 53–75 years, with no history of VTE and not using warfarin, plasma Lp-PLA_2 activity was quite normally distributed with a mean ± SD of 229 ± 62 nmol/min/mL. As reported by others [7,14], Lp-PLA_2 varied markedly by sex and race. Quartile cutpoints for Lp-PLA_2 in nmol/min/mL were: 176, 207, and 242 for white women; 234, 267, and 302 for white men; 147, 178, and 213 for African American women; and 188, 220, and 260 for African American men. We therefore conducted the quartile analyses using sex-race specific quartile cutpoints.

Baseline Lp-PLA_2 was strongly positively correlated with LDL-cholesterol but relatively weakly correlated with the VTE risk factors examined (Table 1). Over a median of 8.3 years of follow-up, we identified 226 VTEs (146 provoked, 80 unprovoked). As reflected by hazard ratios near 1.0 (Table 2), there was little evidence that sex-race specific plasma Lp-PLA_2 activity was an independent risk factor for VTE. Results were largely the same when repeated using overall, rather than sex-race specific, quartile cutpoints (not shown). When treated as a continuous variable, the hazard ratio of total VTE per one standard deviation greater level of Lp-PLA_2 [1.12 (95% CI = 0.94, 1.34)] was nonsignificant (p=0.22), with Model 2 adjustment.

In Model 2 for total VTE, we tested interactions of (a) Lp-PLA_2 by hormone replacement therapy and (b) Lp-PLA_2 by LDL-cholesterol. Neither was statistically significant (p>0.25). Nevertheless, because of potential biological interaction between Lp-PLA_2 and LDL-cholesterol, we examined Lp-PLA_2 associations with VTE, after stratifying participants into two LDL-cholesterol groups (<130 mg/dL or ≥ 130 mg/dL). As shown in Table 3, Lp-PLA_2 was associated positively with VTE in participants with high LDL-cholesterol. However, this was based on small numbers of participants and VTEs within the subgroups, especially in the reference category with high LDL cholesterol and low Lp-PLA_2.

Discussion

In this prospective cohort, greater baseline Lp-PLA_2 activity was not associated overall with an increased incidence of VTE. This contrasts with another inflammatory marker, C-reactive protein, which was associated positively with VTE incidence in this cohort [15]. The lack of an overall association with VTE also contrasts with the consistently reported positive association of Lp-PLA_2 with atherothrombotic events [2–5].

Lp-PLA_2 is a lipid-hydrolyzing enzyme associating primarily with LDL, and LDL could serve as an Lp-PLA_2 substrate [1]. Upon stratification by LDL-cholesterol level, Lp-PLA_2 seemed to be associated positively with VTE occurrence among participants with high LDL-cholesterol. This finding should be viewed with caution because it was a secondary stratified analysis with limited VTE events, and the formal test for statistical interaction between Lp-PLA_2 and LDL-cholesterol was not significant. Yet, it is consistent with higher VTE risk in the setting of both high Lp-PLA_2 and LDL-cholesterol.

Strengths of this study include the moderately large population based biracial sample and the careful risk factor and VTE outcome measures. Drawbacks include (1) the single measure of Lp-PLA_2 activity, (2) no measure of Lp-PLA_2 mass, (3) a sample size that cannot rule out a weak, true association, (4) the relatively long follow-up during which Lp-PLA_2 may have changed, and (5) insufficient data to stratify results by common thrombophilia markers (e.g., Factor V Leiden or prothrombin G20210A) in the full ARIC cohort. It is also remotely possible that an Lp-PLA_2 association with VTE may have been masked by not having Visit 4 data to allow adjustment for diet and physical activity.

In conclusion, our findings, along with one prior study [7], suggest that baseline Lp-PLA_2 is not associated to any significant extent with risk of VTE in the general population. However, further exploration is warranted on whether Lp-PLA_2 may be a VTE risk factor in adults with high LDL-cholesterol.

Acknowledgments

This study was funded by the National Heart, Lung, and Blood Institute grant R01 HL59367 (LITE), and contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C) (ARIC).

The authors thank the staff and participants of the ARIC study for their important contributions over many years.

Abbreviations

ARIC	Atherosclerosis Risk in Communities
Lp-PLA_2	plasma lipoprotein-associated phospholipase A_2
VTE	venous thromboembolism
eGFR	estimate glomerular filtration rate
CRP	C-reactive protein
aPTT	activated partial thromboplastin time
CI	confidence interval
SD	standard deviation
LDL	low-density lipoprotein

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Table 1

Participant characteristics according to sex-race specific quartiles of plasma Lp-PLA$_2$[a] activity at ARIC[b] Visit 4, 1996–98.

	Lp-PLA$_2$ percentile			
Characteristic	**0–25%**	**25–50%**	**50–75%**	**75+ %**
Age, years (SD[c])	62 (6)	62 (6)	63 (6)	63 (6)
Male, n (%)	1174 (44)	1175 (44)	1175 (44)	1175 (44)
African Americans, n (%)	593 (22)	591 (22)	586 (22)	589 (22)
Hormone replacement use in women, n (%)	668 (44)	418 (28)	309 (21)	210 (14)
Body mass index, kg/m^2 (SD)	28.2 (5.8)	28.8 (5.7)	28.9 (5.3)	29.0 (5.3)
Diabetes, n (%)	376 (14)	441 (17)	433 (16)	504 (19)
LDL-cholesterol, mg/dL (SD)	102 (29)	118 (29)	129 (30)	141 (34)
Statin use, n (%)	345 (13)	316 (12)	273 (10)	233 (9)
C-reactive protein, mg/L (SD)	5.1 (7.7)	4.3 (6.0)	4.2 (6.5)	4.2 (5.7)
eGFR[d], ml/min/1.73 m^2 (SD)	85 (20)	82 (20)	80 (19)	77 (19)
Factor VIII,[f] % (SD)	125 (37)	127 (35)	128 (36)	131 (37)
aPTT[e,f], seconds (SD)	29.1 (2.9)	29.0 (3.0)	29.1 (2.9)	29.3 (3.1)

[a] Lp-PLA$_2$: plasma lipoprotein-associated phospholipase A$_2$.

[b] ARIC: Atherosclerosis Risk in Communities.

[c] SD: standard deviation.

[d] eGFR: estimate glomerular filtration rate.

[e] aPTT: activated partial thromboplastin time.

[f] Visit 1, 1987–89 value.

Table 2

Hazard ratio (95% confidence interval) of venous thromboembolism (VTE[a]) incidence according to sex-race specific quartiles of plasma Lp-PLA$_2$[b] activity, ARIC[c], 1996–2005

			Lp-PLA$_2$ percentile				
VTE Endpoint			**0–25%**	**25–50%**	**50–75%**	**75+%**	**P-trend**
Total	Incidence rate		2.6	2.3	2.5	3.1	
	Model 1	N events	57	50	54	65	
		Hazard Ratio (95% CI)	1.00	0.88 (0.60, 1.28)	0.94 (0.65, 1.36)	1.14 (0.80, 1.62)	0.43
	Model 2	N events	53	48	51	59	
		Hazard Ratio (95% CI)	1.00	0.95 (0.64, 1.42)	1.03 (0.69, 1.56)	1.26 (0.83, 1.91)	0.25
Unprovoked	Model 1	N events	23	19	20	18	
		Hazard Ratio (95% CI)	1.00	0.82 (0.45, 1.51)	0.85 (0.47, 1.54)	0.77 (0.41, 1.42)	0.43
	Model 2	N events	20	17	18	18	
		Hazard Ratio (95% CI)	1.00	0.91 (0.47, 1.77)	0.96 (0.48, 1.89)	1.00 (0.49, 2.06)	0.97
Provoked	Model 1	N events	34	31	34	47	
		Hazard Ratio (95% CI)	1.00	0.91 (0.56, 1.48)	0.99 (0.62, 1.60)	1.39 (0.89,2.16)	0.12
	Model 2	N events	33	31	33	41	
		Hazard Ratio (95% CI)	1.00	0.98 (0.59, 1.62)	1.07 (0.64, 1.79)	1.41 (0.84, 2.37)	0.16

[a]VTE: venous thromboembolism.

[b]Lp-PLA$_2$: plasma lipoprotein-associated phospholipase A$_2$.

[c]ARIC: Atherosclerosis Risk in Communities.

[d]VTE rate per 1,000 person-years.

Model 1, adjusted for age, sex and race.

Model 2, adjusted for age, sex and hormone use, race, diabetes status, body mass index, C-reactive protein, eGFR, LDL-cholesterol, and statin use.

Table 3

Hazard ratio[a] (95% confidence interval) of total venous thromboembolism incidence according to sex-race specific quartiles of plasma Lp-PLA$_2$[b] activity, stratified by LDL-cholesterol, ARIC[c], 1996–2005

		Lp-PLA$_2$ percentile				
Stratum		**0–25%**	**25–50%**	**50–75%**	**75+%**	**P-trend**
LDL-cholesterol <130 mg/dL	N[d] at Risk	2102	1651	1311	923	
	N events	49	38	27	25	
	Hazard Ratio (95% CI)	1.00	0.90 (0.58, 1.39)	0.76 (0.47, 1.25)	0.98 (0.58, 1.64)	0.67
LDL-cholesterol ≥ 130 mg/dL	N at Risk	394	836	1161	1536	
	N events	4	10	24	34	
	Hazard Ratio (95% CI)	1.00	1.39 (0.44, 4.44)	2.45 (0.84, 7.11)	2.84 (0.99, 8.14)	0.01

[a] Hazard ratio adjusted for age, sex and hormone use, race, diabetes status, body mass index, C-reactive protein, eGFR, LDL-cholesterol, and statin use.

[b] Lp-PLA$_2$: plasma lipoprotein-associated phospholipase A$_2$.

[c] ARIC: Atherosclerosis Risk in Communities.

[d] N: number.