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Relationship of Lipoprotein-Associated Phospholipase A_2 Mass and Activity with Incident Vascular Events among Primary Prevention Patients Allocated to Placebo or to Statin Therapy: An Analysis from the JUPITER Trial

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BACKGROUND: Although lipoprotein-associated phospholipase A_2 (Lp-PLA_2) levels are associated with cardiovascular events, Lp-PLA_2 is physically linked to LDL cholesterol (LDL-C). Whether measures of Lp-PLA_2 mass or activity continue to predict risk after LDL-C reduction by statin therapy is uncertain.

METHODS: Lp-PLA_2 mass concentration and activity were evaluated at baseline and after treatment in the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial comparing rosuvastatin 20 mg to placebo among 17 802 men and women without cardiovascular disease or diabetes at study entry. The relationships of Lp-PLA_2 mass and activity with risk of future vascular events were evaluated in the placebo and rosuvastatin groups.

RESULTS: Before randomization, levels of Lp-PLA_2 mass and activity correlated moderately with each other and with LDL-C. The magnitude of these correlations increased after statin therapy. Rosuvastatin reduced Lp-PLA_2 mass by 33.8%, Lp-PLA_2 activity by 33.2%, and LDL-C by 48.7% (all $P < 0.0001$). Among those study participants allocated to placebo, increasing quartiles of Lp-PLA_2 activity ($P_{trend} = 0.04$) but not Lp-PLA_2 mass ($P_{trend} = 0.92$) were associated with incident cardiovascular events after adjustment for LDL-C and conventional risk factors. Comparable analyses conducted among those allocated to rosuvastatin revealed no significant relationship between Lp-PLA_2 levels and subsequent vascular events. The ability of rosuvastatin to reduce vascular events was not significantly modified by baseline Lp-PLA_2 level.

CONCLUSIONS: Among JUPITER trial participants allocated to placebo, levels of Lp-PLA_2 activity, but not mass, were associated with cardiovascular risk. However, Lp-PLA_2 no longer predicted risk or modified clinical outcomes when participants were treated with rosuvastatin.

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2)[4] is a proinflammatory enzyme that was found in several cohort studies to be associated with increased risk of vascular events *(1)*. Lp-PLA_2 activity is also the target of darapladib, a potential therapeutic agent under investigation as a treatment administered after acute coronary syndromes and for stable coronary disease *(2, 3)*. However, Lp-PLA_2 is carried in the circulation bound to LDL cholesterol (LDL-C) and apolipoprotein B (apo B) particles *(4)*. Thus, it has been uncertain whether measurements of Lp-PLA_2 mass concentration or activity would continue to predict residual vascular risk after initiation of efficacious statin therapy when LDL-C, non–HDL-C, and apo B are pharmacologically reduced. Furthermore, whether Lp-PLA_2 levels might be useful as a means to select individuals who preferentially benefit from statin therapy is controversial; in the Heart Protection Study, simvastatin reduced Lp-PLA_2 levels by about 25% but the vascular protection associated with simvastatin did not vary with Lp-PLA_2 levels *(5)*. In addition, median levels of Lp-PLA_2 mass measured by using commercial assays for Lp-PLA_2 (PLAC assays) have varied widely in prior epidemiologic studies, making it difficult to use this assay for

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[4] Nonstandard abbreviations: Lp-PLA_2, lipoprotein-associated phospholipase A_2; LDL-C, LDL cholesterol; apo B, apolipoprotein B; JUPITER, Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; hsCRP, high-sensitivity C-reactive protein; HR, hazard ratio.

clinical purposes *(6–11)*, so it has been suggested that measures of Lp-PLA_2 activity might serve as a more reproducible and representative biomarker of enzyme function *(12)*. Indeed, in head-to-head comparisons, Lp-PLA_2 activity has sometimes been a better biomarker of vascular risk than commercially available mass assays *(13, 14)*, whereas in other studies mass and activity have been equivalent *(9–11)*.

We addressed all of these issues by measuring Lp-PLA_2 mass and activity in the recently completed Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial. This trial evaluated daily rosuvastatin 20 mg compared to placebo in the primary prevention of cardiovascular events among men and women with LDL-C <130 mg/dL (<3.37 mmol/L) who were at increased vascular risk on the basis of high-sensitivity C-reactive protein (hsCRP) ≥2 mg/L *(15)*. Our principal aims were to address the level of correlation between Lp-PLA_2 mass and activity with LDL-C among statin treated and untreated patients, and to address whether levels of Lp-PLA_2 mass or activity differentially predicted future vascular events among those patients allocated to placebo compared to those allocated to receive rosuvastatin. We further addressed whether the relative risk reduction attributable to rosuvastatin in the JUPITER trial was modified by the level of either Lp-PLA_2 mass or activity measured at the time of entry of participants into the study.

Methods

The study population derived from JUPITER, a randomized, double-blind, placebo-controlled trial designed to investigate whether rosuvastatin 20 mg daily compared to placebo would decrease the rate of first-ever cardiovascular events among 17 802 apparently healthy men and women with LDL-C <130 mg/dL (<3.37 mmol/L) and hsCRP ≥2 mg/L. Full description of the trial structure and CONSORT (Consolidated Standards of Reporting Trials) diagrams have been presented previously *(15)*, and the trial is registered with ClinicalTrial.gov, number NCT00239681. As reported elsewhere, after a median follow-up of 1.9 years (maximum 5 years), rosuvastatin use was associated with a 54% reduction in myocardial infarction, a 48% reduction in stroke, a 46% reduction in revascularization, a 43% reduction in venous thromboembolism, and a 20% reduction in total mortality *(15, 16)*.

As part of the JUPITER protocol, all study participants provided a blood sample before randomization and after 1 year of therapy with either active rosuvastatin or placebo. These blood samples were assayed in a core laboratory for LDL-C, HDL-C, apo B, and hsCRP as previously described *(15, 17)*. For the current analysis, additional assays for Lp-PLA_2 mass and activity were performed in trial samples obtained prior to and after initiation of statin or placebo therapy. Before and after samples from each trial participant were paired together and stored in liquid nitrogen until the time of assay. Concentrations of Lp-PLA_2 mass were determined by a latex particle–enhanced turbidimetric immunoassay for Lp-PLA_2 run on the Roche P-modular analyzer (PLAC^{TM} test, diaDexus). Lp-PLA_2 activity was measured in a research-use automated enzyme assay system, run on the Roche P-modular analyzer (CAM assay, diaDexus) with a colorimetric substrate that is converted upon hydrolysis by the phospholipase enzyme. Both assays are calibrated to a highly purified recombinant Lp-PLA^2 standard. All assay measurements were performed by the manufacturer on samples that were blinded regarding treatment and outcome results of the testing laboratory.

In accordance with protocol and consistent with prior published analyses for on-treatment hsCRP, on-treatment LDL-C, and on-treatment HDL-C within the JUPITER trial *(17, 18)*, on-treatment levels of Lp-PLA_2 mass and activity were prospectively defined as the values obtained after the first year of treatment. However, as also done in these prior analyses because statins have a maximal impact on lipid levels (and Lp-PLA_2) within 6–8 weeks, we decided a priori to include all postrandomization events in our primary analyses of incident events rather than arbitrarily limiting the analysis to events that occurred after any specific time point. This analytic approach was conservative and clinically relevant, and provided equal follow-up time for study participants randomly allocated to rosuvastatin or to placebo and thus avoided bias that might accrue owing to differential survival or compliance.

Spearman coefficients were used to express the magnitude of correlation between Lp-PLA_2 mass and activity; between these biomarkers and LDL-C, HDL-C, non–HDL-C, and apo B at baseline; and between the change in Lp-PLA_2 and the change in lipid measures after treatment. Following prespecified biomarker analysis plans, we used Cox proportional hazard regression models to calculate hazard ratios (HRs) and 95% CIs for first major cardiovascular events according to increasing baseline quartiles of Lp-PLA_2 levels; these analyses were performed separately and in parallel among those study participants allocated to placebo and among those allocated to rosuvastatin. Regression analyses were initially adjusted for baseline LDL-C, and then multivariable models were used to adjust further for age, sex, smoking status, family history of premature atherosclerosis, body mass index, systolic blood pressure, HDL-C, and hsCRP.

Using similar Cox proportional hazards models, we then calculated HRs and 95% CIs for future vascular events according to increasing quartiles of on-

treatment Lp-PLA_2 mass or activity, again stratifying by placebo or rosuvastatin allocation. For these on-treatment evaluations, on-treatment measurements of LDL-C, HDL-C, and hsCRP were used in the multivariable models. Finally, in subgroups defined by similar quartiles of Lp-PLA_2, we sought evidence for any differential effects of rosuvastatin on vascular event rates during the course of the trial.

All *P* values reported are 2 sided and all CIs were computed at the 95% level. We performed all analyses using the prespecified JUPITER trial primary endpoints, which consisted of the first occurrence of nonfatal myocardial infarction, nonfatal stroke, hospitalization for unstable angina, arterial revascularization, or cardiovascular death. We additionally performed exploratory analyses using the combined primary endpoint plus all-cause mortality. All endpoints were adjudicated by a committee who used standardized diagnostic criteria and were unaware of rosuvastatin or placebo status.

Results

BASELINE AND ON-TREATMENT LEVELS OF LP-PLA_2 MASS AND ACTIVITY

Baseline characteristics of the JUPITER primary prevention study cohort have been described elsewhere in detail *(15)*. Among the 10 439 JUPITER study participants with data for both baseline and 1 year on treatment evaluated in this analysis, the median age of study participants was 66 years, 36% were female, and 41% had metabolic syndrome, and the median baseline concentrations of LDL-C, HDL-C, and hsCRP were 109 mg/dL (2.82 mmol/L), 49 mg/dL (1.27 mmol/L), and 4.1 mg/L, respectively. This distribution of baseline characteristics is almost identical to the JUPITER population as a whole.

Among those allocated to placebo, the baseline median Lp-PLA_2 mass was 301 μg/L and the median Lp-PLA_2 activity was 192 nmol/min/mL. Consistent with prior work, levels of Lp-PLA_2 mass and activity correlated moderately with each other ($r = 0.30$, $P < 0.0001$) and with concentrations of LDL-C, apo B, HDL-C, and non–HDL-C (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol58/issue5).

Those study participants allocated to placebo had a reduction in Lp-PLA_2 mass of 11.7% after 1 year, whereas those allocated to rosuvastatin had a reduction in Lp-PLA_2 mass of 33.8%; this difference between placebo and rosuvastatin was highly significant ($P < 0.0001$). Similarly, those allocated to placebo had a reduction in Lp-PLA_2 activity of 1.7%, whereas those allocated to rosuvastatin had a reduction in Lp-PLA_2 activity of 33.2% ($P < 0.0001$) (see online Supplemental Table 2). After rosuvastatin treatment, the magnitude of the correlation between Lp-PLA_2 mass and activity increased ($r = 0.53$, $P < 0.0001$), as did the magnitude of the correlations between LDL-C and Lp-PLA_2 mass ($r = 0.44$, $P < 0.001$), LDL-C and Lp-PLA_2 activity ($r = 0.57$, $P < 0.001$), and apo B and Lp-PLA_2 activity ($r = 0.62$, $P < 0.001$).

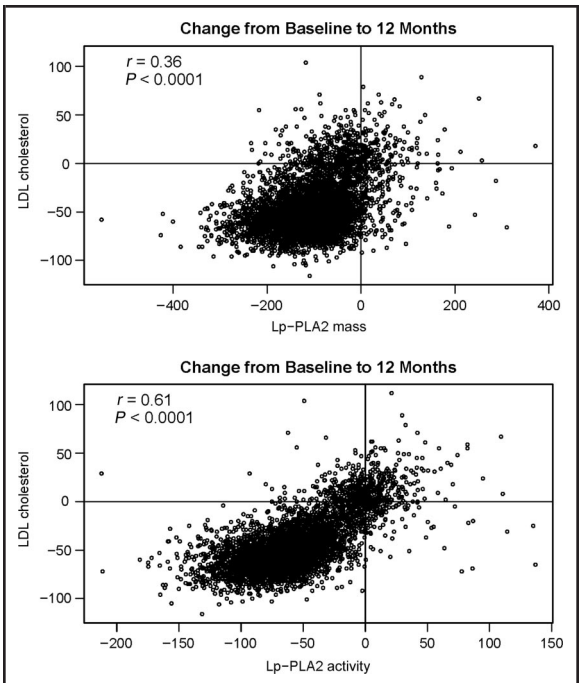

Fig. 1. Relationship of the change in LDL-C and the change in Lp-PLA2 mass (top) and activity (bottom) after treatment with rosuvastatin 20 mg.

As shown in Fig. 1, the change in LDL-C and the change in Lp-PLA_2 mass following rosuvastatin were significantly correlated ($r = 0.36$, $P < 0.001$), as were the change in LDL-C and the change in Lp-PLA_2 activity following rosuvastatin ($r = 0.61$, $P < 0.0001$).

INCIDENT CARDIOVASCULAR EVENTS ACCORDING TO BASELINE LP-PLA_2 LEVELS IN THE PLACEBO AND ROSUVASTATIN GROUPS

Among those allocated to placebo, incidence rates for major cardiovascular events did not significantly increase across increasing baseline quartiles of Lp-PLA_2 mass in models adjusted for LDL-C or in models fully adjusted for either the primary trial endpoint ($P_{trend} = 0.92$) or for the endpoint that included total mortality ($P_{trend} = 0.68$) (Table 1).

In contrast, a significant increase in risk was observed across increasing baseline quartiles of Lp-PLA_2 activity in models adjusted for LDL-C ($P_{trend} = 0.001$) and in models fully adjusted for the primary trial end-

Table 1. Relationship between baseline levels of Lp-PLA$_2$ and all incident vascular events among study participants randomly allocated to placebo.[a]

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*
Lp-PLA$_2$ mass (PLAC assay)					
n	1364	1365	1359	1362	
Median (range)	209.6 (<244.9)	272.7 (245.0–301.3)	333.1 (301.5–366.9)	422.7 (>367.0)	
Primary event					
Events, n	31	38	42	39	
Incidence rate	1.01	1.21	1.34	1.21	
HR$_{\text{adjusted for LDL-C only}}$	1.0	1.18	1.30	1.17	0.53
95% CI	—	0.73–1.89	0.82–2.08	0.73–1.88	
HR$_{\text{fully adjusted}}$	1.0	1.14	1.19	1.00	0.92
95% CI	—	0.71–1.84	0.74–1.91	0.61–1.65	
Primary event plus total mortality					
Events, n	43	55	49	55	
Incidence rate	1.40	1.75	1.56	1.71	
HR$_{\text{adjusted for LDL-C only}}$	1.0	1.25	1.12	1.22	0.47
95% CI	—	0.84–1.86	0.74–1.69	0.82–1.83	
HR$_{\text{fully adjusted}}$	1.0	1.22	0.99	0.99	0.68
95% CI	—	0.81–1.82	0.65–1.51	0.65–1.52	
Lp-PLA$_2$ activity (CAM assay)					
n	1365	1360	1363	1358	
Median (range)	143.3 (<162.8)	177.7 (162.9–191.8)	206.2 (191.9–222.8)	246.0 (>222.9)	
Primary event					
Events, n	17	33	43	56	
Incidence rate	0.57	1.08	1.37	1.66	
HR$_{\text{adjusted for LDL-C only}}$	1.0	1.94	2.41	2.83	0.001
95% CI	—	1.08–3.50	1.36–4.25	1.63–4.94	
HR$_{\text{fully adjusted}}$	1.0	1.74	1.91	2.15	0.04
95% CI	—	0.95–3.19	1.03–3.52	1.13–4.08	
Primary event plus total mortality					
Events, n	32	41	58	70	
Incidence rate	1.07	1.35	1.85	2.08	
HR$_{\text{adjusted for LDL-C only}}$	1.0	1.32	1.78	1.94	0.001
95% CI	—	0.83–2.10	1.15–2.76	1.26–2.98	
HR$_{\text{fully adjusted}}$	1.0	1.17	1.37	1.42	0.16
95% CI	—	0.72–1.89	0.85–2.21	0.86–2.36	

[a] Fully adjusted HRs and 95% CI were adjusted for age, sex, smoking status, family history of premature atherosclerosis, body mass index, and systolic blood pressure as well as baseline levels of LDL-C, HDL-C, and hsCRP. Rates are per 100 person-years. Units for Lp-PLA2 mass are nanograms per liter and for Lp-PLA2 are nanomoles per minute per milliliter.

point ($P_{\text{trend}} = 0.04$). This latter observation for Lp-PLA$_2$ activity remained significant for the endpoint inclusive of total mortality in the LDL-C–adjusted analysis ($P_{\text{trend}} = 0.001$), but not in the fully adjusted analysis ($P_{\text{trend}} = 0.16$) (Table 1).

Table 2 presents comparable data on the relationship of baseline levels of Lp-PLA$_2$ mass and activity with incident major cardiovascular events among those study participants allocated to rosuvastatin. In adjusted analyses, no significant association was observed across increasing quartiles of baseline Lp-PLA$_2$ mass and the primary trial endpoint ($P_{\text{trend}} = 0.92$) or the endpoint inclusive of total mortality ($P_{\text{trend}} = 0.88$). Similarly, as also shown in Table 2 for those allocated to rosuvastatin, no significant association was observed across increasing quartiles of baseline Lp-PLA$_2$ activity

Table 2. Relationship between baseline levels of Lp-PLA$_2$ and all incident vascular events among study participants randomly allocated to rosuvastatin.[a]

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*
Lp-PLA$_2$ mass (PLAC assay)					
n	1379	1372	1377	1373	
Median (range)	209.6 (<242.2)	271.5 (242.3–301.1)	333.4 (301.2–368.0)	422.2 (>368.1)	
Primary event					
Events, n	19	17	25	20	
Incidence rate	0.61	0.54	0.79	0.61	
$HR_{adjusted\ for\ LDL\text{-}C\ only}$	1.0	0.90	1.35	1.04	0.66
95% CI	—	0.46–1.72	0.74–2.47	0.55–1.97	
$HR_{fully\ adjusted}$	1.0	0.79	1.19	0.87	0.92
95% CI	—	0.40–1.56	0.64–2.21	0.44–1.70	
Primary event plus total mortality					
Events, n	29	26	33	35	
Incidence rate	0.93	0.82	1.04	1.07	
$HR_{adjusted\ for\ LDL\text{-}C\ only}$	1.0	0.90	1.18	1.20	0.31
95% CI	—	0.53–1.53	0.71–1.95	0.73–1.99	
$HR_{fully\ adjusted}$	1.0	0.82	1.01	0.97	0.88
95% CI	—	0.48–1.43	0.60–1.69	0.57–1.65	
Lp-PLA$_2$ activity (CAM assay)					
n	1376	1372	1378	1370	
Median (range)	142.6 (<160.8)	175.5 (160.9–190.1)	204.9 (190.2–221.9)	245.5 (>222.0)	
Primary event					
Events, n	18	21	16	25	
Incidence rate	0.61	0.68	0.51	0.73	
$HR_{adjusted\ for\ LDL\text{-}C\ only}$	1.0	1.15	0.86	1.19	0.72
95% CI	—	0.61–2.17	0.43–1.71	1.64–2.22	
$HR_{fully\ adjusted}$	1.0	1.02	0.68	0.83	0.53
95% CI	—	0.52–1.99	0.32–1.44	0.39–1.75	
Primary event plus total mortality					
Events, n	29	26	28	39	
Incidence rate	0.98	0.84	0.89	1.13	
$HR_{adjusted\ for\ LDL\text{-}C\ only}$	1.0	0.88	0.95	1.17	0.44
95% CI	—	0.52–1.51	0.56–1.61	0.71–1.92	
$HR_{fully\ adjusted}$	1.0	0.82	0.78	0.86	0.71
95% CI	—	0.47–1.43	0.43–1.40	0.47–1.55	

[a] Fully adjusted HRs and 95% CI were adjusted for age, sex, smoking status, family history of premature atherosclerosis, body mass index, and systolic blood pressure as well as baseline levels of LDL-C, HDL-C, and hsCRP. Rates are per 100 person-years. Units for Lp-PLA2 mass are nanograms per liter and for Lp-PLA2 are nanomoles per minute per milliliter.

and the primary trial endpoint ($P_{trend} = 0.53$) or the endpoint inclusive of total mortality ($P_{trend} = 0.71$).

INCIDENT CARDIOVASCULAR EVENTS ACCORDING TO ON-TREATMENT Lp-PLA$_2$ LEVELS IN THE PLACEBO AND ROSUVASTATIN GROUP

As shown in Table 3, among those allocated to placebo, we observed no relationship between on-treatment levels of Lp-PLA$_2$ mass and incident vascular events. As observed for baseline values among those allocated to placebo, modest associations between Lp-PLA$_2$ activity and incident vascular events were observed in LDL-C–adjusted analyses, but these effects were attenuated and nonsignificant in fully adjusted analyses.

Finally, as shown in Table 4, among those allocated to rosuvastatin, no significant association was

Table 3. Relationship between on-treatment levels of Lp-PLA$_2$ and all incident vascular events among study participants randomly allocated to placebo.[a]

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*
Lp-PLA$_2$ mass (PLAC assay)					
n	1288	1288	1288	1287	
Median (range)	189.1 (<216.4)	240.7 (216.5–263.7)	290.2 (263.8–322.7)	372.4 (>322.8)	
Primary event					
Events, n	39	37	31	30	
Incidence rate	1.33	1.23	1.06	1.03	
HR$_{\text{adjusted for LDL-C only}}$	1.0	1.03	0.99	1.03	0.93
95% CI	—	0.65–1.62	0.61–1.60	0.63–1.69	
HR$_{\text{fully adjusted}}$	1.0	1.01	0.92	0.89	0.60
95% CI	—	0.64–1.62	0.56–1.51	0.53–1.50	
Primary event plus total mortality					
Events, n	56	47	46	41	
Incidence rate	1.91	1.56	1.57	1.41	
HR$_{\text{adjusted for LDL-C only}}$	1.0	0.90	1.00	0.94	0.89
95% CI	—	0.60–1.33	0.67–1.49	0.62–1.43	
HR$_{\text{fully adjusted}}$	1.0	0.87	0.88	0.75	0.23
95% CI	—	0.59–1.30	0.58–1.32	0.48–1.17	
Lp-PLA$_2$ activity (CAM assay)					
n	1291	1292	1281	1283	
Median (range)	140.0 (<160.0)	174.3 (160.1–187.4)	201.8 (187.5–217.6)	242.1 (>217.7)	
Primary event					
Events, n	30	31	29	46	
Incidence rate	1.07	1.08	0.99	1.44	
HR$_{\text{adjusted for LDL-C only}}$	1.0	1.24	1.25	1.88	0.01
95% CI	—	0.74–2.07	0.73–2.14	1.15–3.06	
HR$_{\text{fully adjusted}}$	1.0	1.10	0.99	1.36	0.27
95% CI	—	0.64–1.87	0.55–1.76	0.77–2.42	
Primary event plus total mortality					
Events, n	44	43	43	59	
Incidence rate	1.57	1.50	1.47	1.85	
HR$_{\text{adjusted for LDL-C only}}$	1.0	1.15	1.23	1.53	0.045
95% CI	—	0.75–1.77	0.79–1.91	1.01–2.32	
HR$_{\text{fully adjusted}}$	1.0	1.01	0.95	1.09	0.72
95% CI	—	0.64–1.57	0.59–1.53	0.67–1.77	

[a] Fully adjusted HRs and 95% CIs were adjusted for age, sex, smoking status, family history of premature atherosclerosis, body mass index, systolic blood pressure, and baseline level of HDL-C as well as on-treatment levels of LDL-C and hsCRP. Rates are per 100 person-years. Units for Lp-PLA2 mass are nanograms per liter and for Lp-PLA2 are nanomoles per minute per milliliter.

observed across increasing quartiles of on-treatment Lp-PLA$_2$ mass and the primary trial endpoint (P_{trend}= 0.89) or the endpoint inclusive of total mortality (P_{trend} = 0.49). For on-treatment Lp-PLA$_2$ activity, a modest relationship with all incident events was observed for those allocated to rosuvastatin across quartiles in LDL-C–adjusted analyses (P_{trend} = 0.07 for the primary trial endpoint and 0.04 for the endpoint inclusive of total mortality). However, these effects were again attenuated and nonsignificant in fully adjusted models (P_{trend} = 0.49 for the primary trial endpoint and 0.47 for the endpoint inclusive of total mortality).

Table 4. Relationship between on-treatment levels of Lp-PLA_2 and all incident vascular events among study participants randomly allocated to rosuvastatin.[a]

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*
Lp-PLA_2 mass (PLAC assay)					
n	1307	1311	1302	1305	
Median (range)	142.3 (<163.9)	181.9 (164.0–199.2)	219.4 (199.4–245.0)	285.3 (>245.1)	
Primary event					
Events, n	14	26	18	22	
Incidence rate	0.47	0.86	0.59	0.76	
$HR_{adjusted\ for\ LDL\text{-}C\ only}$	1.0	1.87	1.21	1.44	0.70
95% CI	—	0.96–3.64	0.58–2.49	0.68–3.04	
$HR_{fully\ adjusted}$	1.0	1.78	1.15	1.21	0.89
95% CI	—	0.88–3.59	0.54–2.46	0.54–2.70	
Primary event plus total mortality					
Events, n	17	33	27	38	
Incidence rate	0.57	1.09	0.88	1.31	
$HR_{adjusted\ for\ LDL\text{-}C\ only}$	1.0	1.91	1.45	1.98	0.10
95% CI	—	1.05–3.47	0.78–2.72	1.05–3.73	
$HR_{fully\ adjusted}$	1.0	1.79	1.33	1.55	0.49
95% CI	—	0.96–3.34	0.69–2.55	0.79–3.06	
Lp-PLA_2 activity (CAM assay)					
n	1309	1301	1301	1303	
Median (range)	93.0 (<106.1)	118.0 (106.2–129.3)	141.8 (129.4–157.6)	182.5 (>157.7)	
Primary event					
Events, n	12	11	30	27	
Incidence rate	0.42	0.37	0.98	0.89	
$HR_{adjusted\ for\ LDL\text{-}C\ only}$	1.0	0.84	2.09	1.70	0.07
95% CI	—	0.37–1.92	1.05–4.15	0.78–3.69	
$HR_{fully\ adjusted}$	1.0	0.67	1.50	1.14	0.49
95% CI	—	0.28–1.57	0.72–3.15	0.48–2.71	
Primary event plus total mortality					
Events, n	18	16	39	42	
Incidence rate	0.63	0.54	1.27	1.38	
$HR_{adjusted\ for\ LDL\text{-}C\ only}$	1.0	0.80	1.73	1.62	0.04
95% CI	—	0.41–1.57	0.97–3.07	0.86–3.05	
$HR_{fully\ adjusted}$	1.0	0.67	1.30	1.09	0.47
95% CI	—	0.33–1.34	0.71–2.41	0.54–2.22	

[a] Fully adjusted HRs and 95% CIs were adjusted for age, sex, smoking status, family history of premature atherosclerosis, body mass index, systolic blood pressure, and baseline level of HDLC as well as on-treatment levels of LDL-C and hsCRP. Rates are per 100 person-years. Units for Lp-PLA2 mass are nanograms per liter and for Lp-PLA2 are nanomoles per minute per milliliter.

EFFECT OF ROSUVASTATIN ON VASCULAR EVENTS ACCORDING TO LEVELS OF Lp-PLA_2 MASS AND ACTIVITY

Rosuvastatin was equally effective at reducing incident vascular events across quartiles of Lp-PLA_2 mass for the endpoint of the main trial and for the endpoint inclusive of total mortality (Fig. 2, top). There was a suggestion of effect modification by baseline level of Lp-PLA_2 activity in that those with baseline Lp-PLA_2 activity in the second, third, and fourth quartile had a somewhat greater relative risk reduction than did those with levels in the first quartile (Fig. 2, bottom). However, the test for interac-

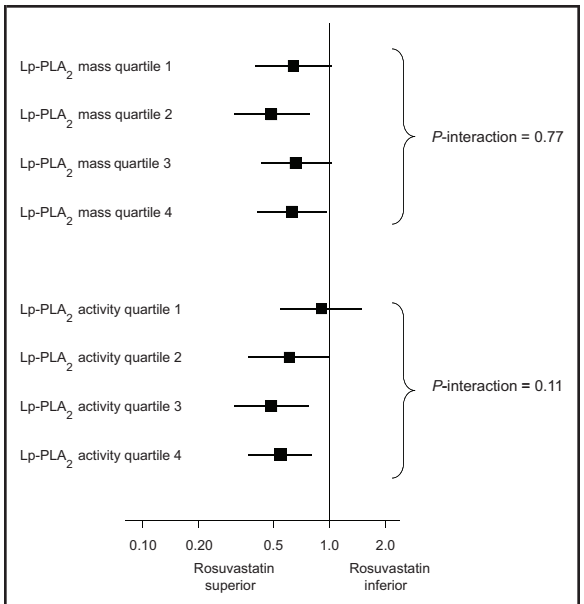

Fig. 2. Efficacy of rosuvastatin as compared to placebo on the JUPITER trial primary endpoint and on the endpoint inclusive of total mortality, according to baseline levels of Lp-PLA2 mass and activity.

tion for this difference was not statistically significant ($P = 0.11$).

Discussion

In this evaluation of the JUPITER trial population, we observed that the level of Lp-PLA_2 activity, but not Lp-PLA_2 mass, was a predictor of future cardiovascular events among those study participants allocated to placebo. These data are of pathophysiologic interest because Lp-PLA_2 activity is the target of the specific molecular inhibitor darapladib, which is currently under investigation in both acute coronary syndrome and chronic coronary artery disease patients *(2, 3)*. Thus, our data among those study participants allocated to placebo support the concept of inhibiting Lp-PLA_2 as a potential therapeutic target. However, our data also provide a cautionary note for trials of Lp-PLA_2 inhibition because among those study participants allocated to efficacious statin therapy, Lp-PLA_2 levels were no longer significantly associated with cardiovascular events in the JUPITER population [in which concentrations of LDL-C were decreased to approximately 50 mg/dL (1.30 mmol/L)]. In part, as shown in Fig. 1, this lack of association may reflect the high correlation between on-treatment changes in LDL-C and on-treatment changes in Lp-PLA_2 activity.

In the current analyses, we also observed that rosuvastatin was equally effective among those study participants with lower as well as higher baseline levels of Lp-PLA_2 mass. Thus, in these data, in a population with increased hsCRP concentrations at baseline, there was no clinical utility for evaluation of Lp-PLA_2 mass either for risk prediction or for statin targeting. For Lp-PLA_2 activity, the point estimates of effect for rosuvastatin benefit in the second, third, and fourth quartile were somewhat greater than those in the first quartile, but this difference was not statistically significant. These null data are consistent with prior work from the Heart Protection Study of simvastatin, in which no evidence for effect modification was observed for baseline levels of Lp-PLA_2 mass or activity *(5)*. That Lp-PLA_2 levels did not modify the benefit of statin therapy may again reflect tight linkage between apo B, LDL-C, non–HDL-C, and Lp-PLA_2. In prior work for simvastatin, pravastatin, rosuvastatin, ezetimibe, and fenofibrate *(5, 19, 20)*, reductions in Lp-PLA_2 have been attributable in part to concomitant reductions in LDL-C or apo B.

At first glance, the fact that levels of Lp-PLA_2 mass in our study did not predict future events may seem surprising because positive effects were reported for several prior studies. For example, in a collaborative analysis of 32 prior prospective studies, each SD increase in Lp-PLA_2 mass was associated with an increase in relative risk of 1.11 for coronary heart disease (95% CI 1.07–1.24) *(1)*. On the other hand, in that meta-analysis, not all prior studies were positive, nor did all assays used for the measurement of Lp-PLA_2 mass perform similarly. Furthermore, because substantial variation in measured levels was observed between studies, the metaanalysis required the use of normalized Lp-PLA_2 values that, although biologically informative, greatly limit clinical application. These data highlight the complexity of using Lp-PLA_2 mass in clinical practice. Although commercial algorithms for the use of $LpPLA_2$ mass indicate that patient values above or below 200 μg/L have clinical relevance for risk prediction, the median value in the current data was much higher than this value (301 μg/L), and several other epidemiologic studies also revealed median values well above this clinical cutpoint. As recent examples, the median Lp-PLA_2 mass levels in the CARDIA (Coronary Artery Risk Development in young Adults), PROSPER (PROspective Study of Pravastatin in the Elderly at Risk), CHS (Cardiovascular Health Study), ARIC (Atherosclerosis Risk in Communities), and Rancho-Bernardo cohorts were 267, 300, 335, 373, and 494 μg/L, respectively *(6–8, 10, 11)*. The finding of such wide variation of median levels for Lp-PLA_2 mass across many populations presents a substantive calibration issue that could further limit the use of Lp-

PLA_2 mass in clinical practice. Our positive data for Lp-PLA_2 activity suggest that this alternative approach to measuring enzyme function may be more promising. Other studies have also demonstrated Lp-PLA_2 activity to be a better predictor than mass *(13, 14)*.

Limitations of our analysis merit consideration. First, because Lp-PLA_2 is physically linked with apo B, controlling our risk ratios for LDL-C (or apo B) may be an overadjustment, at least in terms of understanding pathophysiology. Partly for this reason, we believe trials of Lp-PLA_2 inhibition remain important. On the other hand, because LDL-C is routinely measured after statin initiation and is a predictor of residual risk, our adjusted analyses suggest little clinical utility for the additional evaluation of Lp-PLA_2 in that setting, in particular when concentrations of LDL-C <70 mg/dL (<1.81 mmol/L) are achieved.

Second, although a major strength of our study is its randomized placebo-controlled design, the entry criteria for JUPITER constrain the absolute ranges for LDL-C and hsCRP; as such, the current data may not be fully generalizable. However, the fact that neither Lp-PLA_2 mass nor activity modified the effect of simvastatin in the Heart Protection Study *(5)* suggests that this limitation is unlikely to have altered our main findings.

Third, loss-of-function polymorphisms for Lp-PLA_2 activity have been reported in Japanese, Chinese, and Korean populations and have been associated with reduced Lp-PLA_2 activity *(21–24)*. The JUPITER trial, however, did not enroll study participants in Asia so these effects could not have had an impact on our data. Results of recent genome-wide studies suggest that common polymorphisms in Western populations can impact modestly on plasma Lp-PLA_2 activity levels *(25)*, although these latter effects have not been linked to differential event rates. A genome-wide evaluation of Lp-PLA_2 within the JUPITER trial is underway and may shed further light on this issue.

Fourth, an 11.7% reduction in Lp-PLA_2 mass was observed in the placebo group between study entry and 1 year of participation ($P < 0.001$) despite no clinical intervention other than enrollment in a clinical trial. This finding is unlikely to represent a "healthy cohort effect" because LDL-C concentrations did not drop significantly during this time frame among those study participants allocated to placebo and because the change in Lp-PLA_2 activity in these same samples was only 1.7%. As such, we cannot exclude the possibility that this change reflects added assay imprecision in the measurement of Lp-PLA_2 mass.

Finally, as in any null study, power must be considered. We believe it unlikely that power in JUPITER is insufficient to find a true association between on-treatment levels of LpPLA_2 and subsequent vascular events, because prior analyses of these data have shown on-treatment levels of LDL-C, apo B, and hsCRP to significantly predict residual risk *(17, 18)*.

In sum, within this large randomized trial of rosuvastatin conducted in primary prevention, levels of Lp-PLA_2 activity, but not mass, were modestly associated with cardiovascular risk among those study participants randomly allocated to placebo. However, among those randomly allocated to rosuvastatin 20 mg daily, Lp-PLA_2 levels no longer predicted risk nor modified clinical outcomes. These findings have implications both for clinical practice and for ongoing trials of Lp-PLA_2 inhibition.

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