ORIGINAL RESEARCH

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Changes in Lipoprotein-Associated Phospholipase A2 Activity Predict Coronary Events and Partly Account for the Treatment Effect of Pravastatin: Results From the Long-term Intervention with Pravastatin in Ischemic Disease Study

Harvey D. White, DSc;* John Simes, MD;* Ralph A. H. Stewart, MD; Stefan Blankenberg, MD; Elizabeth H. Barnes, MStat; Ian C. Marschner, PhD; Peter Thompson, MD; Malcolm West, MD; Tanja Zeller, PhD; David M. Colquhoun, MD; Paul Nestel, MD; Anthony C. Keech, MD; David R. Sullivan, MD; David Hunt, MD; Andrew Tonkin, MD; for the LIPID Study Investigators

***Background*—**Lipoprotein-associated phospholipase A2 (Lp-PLA_2) levels are associated with coronary heart disease (CHD) in healthy individuals and in patients who have had ischemic events.

***Methods and Results*—**The Long-term Intervention with Pravastatin in Ischemic Disease (LIPID) study randomized 9014 patients with cholesterol levels of 4.0 to 7.0 mmol/L to placebo or pravastatin 3 to 36 months after myocardial infarction or unstable angina and showed a reduction in CHD and total mortality. We assessed the value of baseline and change in Lp-PLA_2 activity to predict outcomes over a 6-year follow-up, the effect of pravastatin on Lp-PLA_2 levels, and whether pravastatin treatment effect was related to Lp-PLA_2 activity change. Lp-PLA_2 was measured at randomization and 1 year, and levels were grouped as quartiles. The prespecified end point was CHD death or nonfatal myocardial infarction. Baseline Lp-PLA_2 activity was positively associated with CHD events ($P<0.001$) but not after adjustment for 23 baseline factors ($P=0.66$). In 6518 patients who were event free at 1 year, change in Lp-PLA_2 was a significant independent predictor of subsequent CHD events after adjustment for these risk factors, including LDL cholesterol and LDL cholesterol changes ($P<0.001$). Pravastatin reduced Lp-PLA_2 by 16% compared with placebo ($P<0.001$). After adjustment for Lp-PLA_2 change, the pravastatin treatment effect was reduced from 23% to 10% ($P=0.26$), with 59% of the treatment effect accounted for by changes in Lp-PLA_2. Similar reductions in treatment effect were seen after adjustment for LDL cholesterol change.

***Conclusion*—**Reduction in Lp-PLA_2 activity during the first year was a highly significant predictor of CHD events, independent of change in LDL cholesterol, and may account for over half of the benefits of pravastatin in the LIPID study. **(*J Am Heart Assoc.* 2013;2:e000360 doi: 10.1161/JAHA.113.000360)**

Key Words: biomarkers • LIPID • Lp-PLA_2 • pravastatin

Products of the enzyme lipoprotein-associated phospholipase A2 (Lp-PLA_2) are found in plaques and are proinflammatory and proapoptotic.[1] Lp-PLA_2 is also a marker of vulnerable plaques.[2,3] Elevated plasma Lp-PLA_2 levels are associated with increased coronary heart disease (CHD) risk in both healthy individuals and patients who have already had coronary events.[4]

The Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) study was a large double-blind, placebo-controlled study of pravastatin in stable CHD patients.[5] During a mean 6.0-year follow-up, there were significant reductions in the primary end point of death from CHD, all-cause mortality, and other prespecified cardiovascular end points, including the composite of nonfatal myocardial infarction (MI) or CHD death. There was a reduction in death from CHD death by 24% ($P<0.001$) and overall mortality by 22% ($P<0.001$). Nonfatal MI or death due to CHD was reduced by 24%.

From the Green Lane Cardiovascular Service, Auckland City Hospital, Auckland, New Zealand (H.D.W., R.A.H.S.); NHMRC Clinical Trials Centre, University of Sydney, Sydney, Australia (J.S., E.H.B., I.C.M., A.C.K.); University Heart Centre Hamburg, Hamburg, Germany (S.B., T.Z.); University of Western Australia, Perth, Australia (P.T.); University of Queensland, Brisbane, Australia (M.W.); Greenslopes Hospital, Greenslopes, Australia (D.M.C.); Baker IDI Heart and Diabetes Institute, Melbourne, Australia (P.N.); Royal Prince Alfred Hospital, Camperdown, Australia (D.R.S.); Department of Cardiology and University of Melbourne, Melbourne, Australia (D.H.); Monash University, Melbourne, Australia (A.T.); Department of Statistics, Macquarie University, Sydney, Australia (I.C.M.); for the LIPID Study Investigators.

*Drs White and Simes are joint first authors.

Correspondence to: Harvey White, Green Lane Cardiovascular Service, Auckland City Hospital, Private Bag 92024, Victoria St West, Auckland 1142, New Zealand. E-mail: HarveyW@adhb.govt.nz

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Reduction in LDL cholesterol (LDL-C) is thought to be the major mechanism by which statins reduce risk. It is possible that other mechanisms are also important. Statins have been shown to reduce Lp-PLA$_2$ levels by up to 33%,[6,7,15,16] and therefore some of the benefits of pravastatin could be explained by this effect. We therefore assessed in a prespecified analysis of the LIPID study data the predictive value of baseline and changes in Lp-PLA$_2$ for major coronary events (CHD death or nonfatal MI), the effect of pravastatin on Lp-PLA$_2$ levels, and the extent to which pravastatin treatment effect might be explained by effects on Lp-PLA$_2$.

Methods

Study Design and Patients

The design and major results of the LIPID study have been described in detail previously.[5] A total of 9014 patients aged 31 to 75 years (7498 men, 1516 women), with an MI or hospital discharge diagnosis of unstable angina 3 to 36 months previously, were enrolled on the study if their plasma total cholesterol was 4.0 to 7.0 mmol/L (155 to 271 mg/dL), fasting triglycerides were <445 mg/dL (<5.0 mmol/L), and they satisfied other broad inclusion and exclusion criteria. After informed consent and an 8-week placebo run-in phase to assess compliance, patients were randomly allocated to receive pravastatin 40 mg daily or matching placebo. Patients were otherwise treated with standard therapies.

The primary prespecified outcome for substudies was a composite of CHD death and nonfatal MI. MI was diagnosed by the presence of at least 2 new pathologic Q waves on the electrocardiogram or 2 of the following 3 criteria: ≥15 minutes of ischemic chest pain, evolutionary ST-T wave changes, or elevation of the serum level of creatine kinase or its MB isoenzyme to at least twice the upper limit of normal. All deaths and MIs were reviewed by an Outcomes Assessment Committee whose members had no knowledge of the patient's treatment assignment.

The trial and biomarker analyses were conceived, managed, and analyzed independently of the sponsor.

Laboratory Methods and Biomarkers

Blood was drawn from consenting participants at baseline before treatment allocation after a 12-hour fast into EDTA tubes, and plasma was stored at −70°C. Biomarkers including Lp-PLA$_2$ were analyzed using baseline samples from 87% of patients (n=7863) and 86% (n=6657) of patients alive at 1 year.

Lp-PLA$_2$ activity was measured in a research-use automated enzyme assay system (CAM Assay; DiaDexus Inc), run on the Abbott Architect c8000 analyzer, using a colorimetric PAF analog substrate that is converted on hydrolysis by the phospholipase enzyme. The assay is calibrated to a highly purified recombinant Lp-PLA$_2$ standard. Intra-assay coefficient of variation (CV) was 1.2%, and interassay CV was 3.8%. All assay measurements were performed by laboratory personnel who were blinded to the study samples regarding treatment assignment and outcomes.

Fasting serum lipids (total cholesterol, HDL cholesterol, and triglycerides) were measured in a central laboratory at Flinders Medical Centre, Adelaide, South Australia, a World Health Organization reference center. LDL cholesterol was calculated using the Friedewald formula.[8]

Statistics

Analyses were prespecified in a biomarker protocol. Baseline Lp-PLA$_2$ activity and changes to 1 year were grouped as quartiles. Hazard ratios (HRs) and 95% CIs were estimated using Cox proportional-hazards regression models. Analyses were adjusted for sex, pravastatin treatment, and 22 other traditional risk factors. The primary end point for biomarker analyses was a composite of CHD death and nonfatal MI. Secondary end points included major cardiovascular disease (CVD) outcomes (a composite of CVD death, MI, and stroke) and total CVD outcomes (a composite of major CVD events, unstable angina, and coronary revascularization).

The relationship between Lp-PLA$_2$ activity and CVD outcomes was assessed using a prespecified time to event model adjusted for sex, randomized treatment, prior stroke, diabetes mellitus, current smoking, hypertension, fasting glucose, total cholesterol, apolipoprotein B, apolipoprotein A1, HDL cholesterol, triglycerides, age, nature of prior acute coronary syndromes, timing of coronary revascularization, systolic blood pressure, atrial fibrillation, estimated glomerular filtration rate, body mass index, dyspnea class, angina grade, white blood cell count, peripheral vascular disease, and use of aspirin at baseline.

The relationship between changes in Lp-PLA$_2$ activity from baseline to 1 year and subsequent CVD events was assessed in a landmark analysis using Cox regression in a model that incorporated the standard risk factors previously indicated as well as baseline Lp-PLA$_2$ activity. We also performed backward selection for a landmark model looking at events from 1 year adjusted for baseline brain natriuretic peptide, baseline cystatin C, baseline D-dimer, baseline troponin I, baseline Lp-PLA$_2$ activity, and change in these same biomarkers.

The extent to which the pravastatin effect on subsequent CVD events was accounted for by changes in Lp-PLA$_2$ activity and changes in LDL-C was assessed by estimating the

relative risk reduction from pravastatin before and after adjustment for these changes as continuous variables in these Cox regression models.[9] A sensitivity analysis of treatment effect accounted for by changes in Lp-PLA$_2$ activity and LDL-C used change in these variables grouped in quartiles.

Results

Of the 9014 patients randomized in LIPID, 7863 patients had baseline measurement of Lp-PLA$_2$ levels and formed the cohort for this study (Figure 1). The baseline characteristics of patients included and of those not included in this analysis are shown in Table 1.

Baseline characteristics of the patients divided into the quartiles (<229, 229 to 261, 261 to 294, >294 nmoL/min per milliliter) of Lp-PLA$_2$ activity are shown in Table 2. Lp-PLA$_2$ activity was significantly lower in females (Figure 2) and with lower LDL-C, lower triglycerides, or higher HDL cholesterol levels. There were higher associations with a history of multiple MIs, higher LIPID risk score,[10] higher WBC, cystatin C, and lower estimated glomerular filtration rate. Lp-PLA$_2$ activity was not associated with high-sensitivity C-reactive protein or troponin I.

Relationship Between Lp-PLA$_2$ and Clinical Events

Higher baseline Lp-PLA$_2$ activity was associated with an increased risk of CHD events, major CVD events, total CVD events, CHD death, and all-cause mortality significant (each $P \leq 0.01$). After adjustment for all baseline factors, there was no longer a significant association between Lp-PLA$_2$ activity and clinical events with the exception of CHD death ($P=0.05$) (Figure 3).

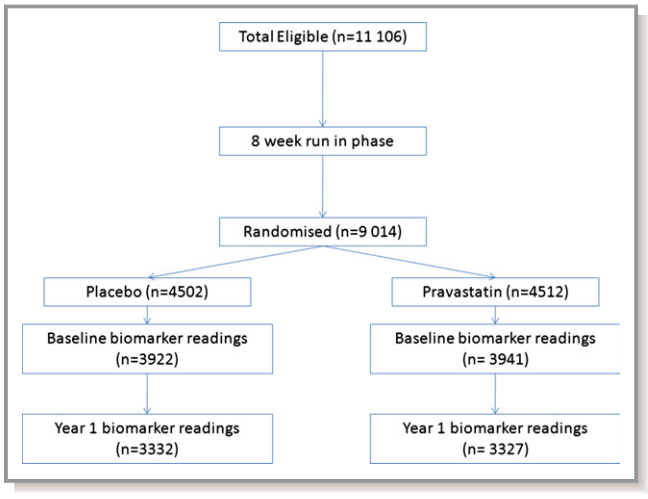

Figure 1. Consort diagram for patient flow.

Effect of Pravastatin on Lp-PLA$_2$ Levels and CVD Events

Lp-PLA$_2$ activity levels were reduced by 16% (262 nmoL/min per milliliter versus 218 nmoL/min per milliliter) in the pravastatin group at 12 months while levels decreased by 0.4% in the placebo group ($P<0.001$) (Figures 4 and 5). Figures 6 and 7 show the relationship between levels of Lp-PLA$_2$ levels and on treatment LDL-C at baseline and changes to year 1. Effects of pravastatin on clinical events by each quartile of Lp-PLA$_2$ activity are shown in Table 3. Pravastatin resulted in a significant reduction in CHD events, CVD events, CHD death, and all-cause mortality with no significant variation in treatment effect according to baseline Lp-PLA$_2$ activity. The numbers needed to treat to prevent 1 event were lower in patients with higher Lp-PLA$_2$ activity, but there was no significant trend in the effect of pravastatin across the quartiles of baseline Lp-PLA$_2$ activity.

Relationship Between Change in Lp-PLA$_2$ Activity and Events

Figure 8 shows the association of change of Lp-PLA$_2$ over 1 year with subsequent CVD events. A larger decrease in Lp-PLA$_2$ was associated with fewer CHD events ($P<0.002$), major CVD events ($P=0.003$), and total CVD events ($P=0.001$) after adjustment for baseline factors. In a landmark model looking at events after 1 year and adjusting for baseline brain natriuretic peptide, baseline cystatin C, baseline D-dimer, baseline troponin I, baseline Lp-PLA$_2$ activity, and change in these same biomarkers, there was still a strong association with change in Lp-PLA$_2$ activity and CHD events ($P<0.001$) (Table 4). Further models were fitted to examine the strength of the association between change in Lp-PLA$_2$ activity and events after year 1. These models were not prespecified but were included as a sensitivity analysis. They showed that when baseline and change in LDL-C were included in the model and/or when baseline and change in Lp-PLA$_2$ and LDL-C were fitted as continuous variables rather than quartiles, the strong association between change in Lp-PLA$_2$ and CHD events was maintained, while change in LDL-C was not a predictor of outcomes (data not shown).

Extent of Pravastatin Treatment Effect Explained by Change in Lp-PLA$_2$

Figure 9 shows the effect of pravastatin versus placebo on CVD events before and after adjustment for changes in Lp-PLA$_2$ activity and LDL-C. Pravastatin was associated with a 23% reduction in CHD events after adjustment for all baseline risk factors ($P<0.001$). After adjustment for change in LDL-C,

Table 1. Baseline Characteristics of Patients in the Biomarker Analysis vs Others

	Patients With All Biomarker Readings at Baseline	All Other Patients	
	7863 (100%)	1151 (100%)	*P* Value*
Pravastatin, No. (%)	3941 (50)	571 (50)	
Age at randomization, y, median (IQR)	62.0 (55.0 to 67.0)	61.0 (54.0 to 67.0)	<0.001
Age ≥65 y, No. (%)	3109 (40)	405 (35)	<0.01
Female, No. (%)	1333 (17)	183 (16)	0.37
Baseline health			
Months from QE, median (IQR)	13.9 (7.9 to 25.0)	12.8 (7.6 to 24.0)	0.03
Current smoker, No. (%)	735 (9)	134 (12)	0.01
Hypertension, No. (%)	3291 (42)	467 (41)	0.41
Diabetes, No. (%)	676 (9)	106 (9)	0.49
Obese, No. (%)	1397 (18)	214 (19)	0.50
Stroke, No. (%)	322 (4)	47 (4)	0.99
Atrial fibrillation, No. (%)	110 (1)	13 (1)	0.46
Systolic BP, mean (SD), mm Hg	134 (19)	133 (18)	0.11
Diastolic BP, mean (SD), mm Hg	81 (11)	80 (11)	0.64
Dyspnea NYHA Class >1, No. (%)	761 (10)	97 (8)	0.18
Angina CCS Grade >0, No. (%)	2927 (37)	401 (35)	0.12
Baseline lipids			
Total cholesterol, mean (SD), nmol/L	5.7 (0.8)	5.6 (0.8)	0.03
Total cholesterol ≥5.5 mmol/L, No. (%)	4495 (57)	639 (56)	0.29
LDL-C, mean (SD), nmol/L	3.9 (0.7)	3.8 (0.7)	<0.01
LDL-C ≥3.5 mmol/L, No. (%)	5513 (70)	780 (68)	0.11
HDL-C, mean (SD), nmol/L	1.0 (0.2)	1.0 (0.2)	0.84
HDL-C ≥1 mmol/L, No. (%)	2936 (37)	422 (37)	0.67
Triglyceride, median (IQR), nmol/L	1.6 (1.2 to 2.2)	1.6 (1.2 to 2.2)	0.39
Total cholesterol:HDL-C, mean (SD), nmol/L	6.2 (1.5)	6.2 (1.5)	0.37
eGFR, median (IQR), mLs/min	69 (60 to 80)	70 (60 to 81)	0.56
WBC, median (IQR) $\times 10^9$	7.0 (6.0 to 8.2)	7.1 (6.0 to 8.3)	0.30
Previous coronary revascularization			
No revascularization, No. (%)	4610 (59)	712 (62)	0.03
PCI only, No. (%)	870 (11)	118 (10)	—
CABG only, No. (%)	2141 (27)	295 (26)	—
PCI and CABG, No. (%)	242 (3)	26 (2)	—
Qualifying Event			
No MI, No. (%)	2843 (36)	417 (36)	0.90
Single MI, No. (%)	4115 (52)	604 (52)	—
Multiple MI, No. (%)	905 (12)	130 (11)	—
Medications			
Aspirin, No. (%)	6501 (83)	901 (78)	<0.001
ACE inhibitors, No. (%)	1254 (16)	179 (16)	0.73
β-Blocker, No. (%)	3691 (47)	538 (47)	0.90
Calcium antagonist, No. (%)	2688 (34)	424 (37)	0.08
LIPID risk score, mean (SD)[10]	5.8 (3.5)	6.0 (3.5)	0.26

No. (%) is presented unless otherwise stated. ACE indicates angiotensin-converting enzyme; BP, blood pressure; CABG, coronary artery bypass graft surgery; CCS, Canadian Cardiovascular Society; eGFR, estimated glomerular filtration rate; HDL-C, HDL cholesterol; IQR, interquartile range; LDL-C, LDL cholesterol; LIPID, Long-term Intervention with Pravastatin in Ischaemic Disease; MI, myocardial infarction, NYHA, New York Heart Association; PCI, percutaneous coronary intervention; WBC, white blood cell count.
**P*-values for continuous variables are from a linear model, and for categorical variables are from an ordinal or logistic regression.

Table 2. Baseline Risk Factors by Lp-PLA$_2$ Activity Categories

	Lp-PLA$_2$ Activity ≤229 nmol/min per milliliter	Lp-PLA$_2$ Activity 229 to 261 nmol/min per milliliter	Lp-PLA$_2$ Activity 261 to 294 nmol/min per milliliter	Lp-PLA$_2$ Activity >294 nmol/min per milliliter	*P* Value Trend*
	1970	1962	1965	1966	
Lp-PLA$_2$ activity, mean (SD), nmol/min per milliliter	199.4 (25.4)	245.8 (9.1)	276.8 (9.4)	326.0 (29.5)	
Age at randomization, median (IQR), y	63.0 (56.0 to 68.0)	63.0 (56.0 to 68.0)	62.0 (55.0 to 67.0)	62.0 (55.0 to 67.0)	<0.01
Age ≥65 y, No. (%)	798 (41)	819 (42)	733 (37)	759 (39)	0.06
Female, No. (%)	658 (33)	364 (19)	195 (10)	116 (6)	<0.001
Allocated pravastatin, No. (%)	990 (50)	996 (51)	981 (50)	974 (50)	0.57
Baseline health					
Months from QE, median (IQR)	13.4 (7.5 to 24.9)	13.5 (7.6 to 24.6)	14.3 (8.0 to 25.2)	14.5 (8.3 to 25.3)	0.05
Current smoker, No. (%)	156 (8)	181 (9)	183 (9)	215 (11)	<0.01
Hypertension, No. (%)	877 (45)	818 (42)	801 (41)	795 (40)	<0.01
Diabetes mellitus, No. (%)	201 (10)	167 (9)	152 (8)	156 (8)	<0.01
Obese, No. (%)	357 (18)	354 (18)	340 (17)	346 (18)	0.56
Previous stroke, No. (%)	83 (4)	74 (4)	78 (4)	87 (4)	0.68
Atrial fibrillation, No. (%)	21 (1)	20 (1)	30 (2)	39 (2)	<0.01
Systolic BP, mean (SD), mm Hg	135 (19)	135 (19)	134 (19)	134 (20)	0.11
Diastolic BP, mean (SD), mm Hg	80 (11)	81 (11)	81 (11)	81 (11)	0.32
Dyspnea, No. (%) NYHA Class >1, No. (%)	205 (10)	171 (9)	163 (8)	222 (11)	0.40
Angina CCS Grade >0, No. (%)	727 (37)	681 (35)	711 (36)	808 (41)	<0.01
Baseline lipids					
Total cholesterol, mean (SD), mmol/L	5.5 (0.9)	5.6 (0.8)	5.7 (0.8)	5.8 (0.8)	<0.001
LDL-C, mean (SD)	3.6 (0.7)	3.9 (0.7)	4.0 (0.7)	4.1 (0.7)	<0.001
HDL-C, mean (SD)	1.1 (0.3)	1.0 (0.2)	0.9 (0.2)	0.8 (0.2)	<0.001
Triglycerides, median (IQR)	1.4 (1.1 to 2.0)	1.5 (1.1 to 2.1)	1.6 (1.2 to 2.2)	1.7 (1.3 to 2.3)	<0.001
Total cholesterol:HDL-C, mean (SD)	5.3 (1.4)	6.0 (1.3)	6.4 (1.3)	7.1 (1.5)	<0.001
Previous coronary revascularization					
No revascularization, No. (%)	1197 (61)	1145 (58)	1145 (58)	1123 (57)	<0.001
PCI only, No. (%)	259 (13)	221 (11)	230 (12)	160 (8)	—
CABG only, No. (%)	453 (23)	531 (27)	532 (27)	625 (32)	—
PCI and CABG, No. (%)	61 (3)	65 (3)	58 (3)	58 (3)	—
Qualifying Event					
No MI, No. (%)	762 (39)	703 (36)	687 (35)	691 (35)	<0.001
Single MI, No. (%)	1025 (52)	1042 (53)	1047 (53)	1001 (51)	—
Multiple MI, No. (%)	183 (9)	217 (11)	231 (12)	274 (14)	—
Medications					
Aspirin, No. (%)	1611 (82)	1631 (83)	1666 (85)	1593 (81)	0.78
ACE inhibitors, No. (%)	310 (16)	297 (15)	297 (15)	350 (18)	0.08
β-Blocker, No. (%)	928 (47)	896 (46)	961 (49)	906 (46)	0.92
Calcium antagonist, No. (%)	701 (36)	664 (34)	636 (32)	687 (35)	0.52

Continued

Table 2. Continued

	Lp-PLA$_2$ Activity ≤229 nmol/min per milliliter	Lp-PLA$_2$ Activity 229 to 261 nmol/min per milliliter	Lp-PLA$_2$ Activity 261 to 294 nmol/min per milliliter	Lp-PLA$_2$ Activity >294 nmol/min per milliliter	*P* Value Trend*
	1970	1962	1965	1966	
LIPID risk score,[10] mean (SD)	4.9 (3.4)	5.7 (3.4)	6.0 (3.4)	6.7 (3.5)	<0.001
Baseline biomarker levels					
eGFR, median (IQR), mL/min	70 (60 to 81)	70 (61 to 80)	70 (61 to 80)	69 (59 to 80)	<0.001
WBC, median (IQR), $\times 10^9$	6.9 (5.9 to 8.1)	6.9 (5.9 to 8.1)	7.1 (6.0 to 8.2)	7.2 (6.1 to 8.5)	<0.001
BNP, median (IQR), pg/mL	23.9 (10.0 to 51.9)	23.3 (9.7 to 50.1)	23.1 (10.0 to 49.2)	23.6 (9.6 to 52.1)	0.03
Troponin I, median (IQR), ng/mL	0.011 (0.006 to 0.021)	0.010 (0.006 to 0.019)	0.010 (0.006 to 0.021)	0.011 (0.006 to 0.021)	0.55
CRP, median (IQR), mg/L	2.5 (1.2 to 5.0)	2.4 (1.2 to 4.9)	2.4 (1.2 to 4.6)	2.5 (1.3 to 4.8)	0.15
Cystatin C, median (IQR), mg/L	0.78 (0.70 to 0.89)	0.80 (0.72 to 0.91)	0.81 (0.73 to 0.93)	0.85 (0.75 to 0.97)	<0.001
D-dimer, median (IQR), ng/L	168 (109 to 266)	167 (109 to 270)	174 (112 to 276)	183 (117 to 278)	0.63
LP(a), median (IQR), mg/L	13.5 (6.4 to 39.6)	13.7 (6.5 to 43.8)	14.3 (6.7 to 46.3)	14.1 (6.8 to 46.7)	<0.01
Mid regional proadrenomedullin, median (IQR), nmol/L	0.47 (0.37 to 0.57)	0.47 (0.38 to 0.58)	0.48 (0.39 to 0.57)	0.48 (0.39 to 0.59)	0.02

No. (%) is presented unless otherwise stated. BNP indicates brain natriuretic peptide; BP, blood pressure; CABG, coronary artery bypass graft surgery; CCS, Canadian Cardiovascular Society; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL-C, HDL cholesterol; IQR, interquartile range; LDL-C, LDL lipoprotein cholesterol; LIPID, Long-term Intervention with Pravastatin in Ischaemic Disease; LP, lipoprotein; Lp-PLA$_2$, lipoprotein-associated phospholipase A2; MI, myocardial infarction, NYHA, New York Heart Association; PCI, percutaneous coronary intervention; WBC, white blood cell count.
**P*-values for trend for continuous variables are from generalized linear models and, for categorical variables, are from an ordinal or logistic regression.

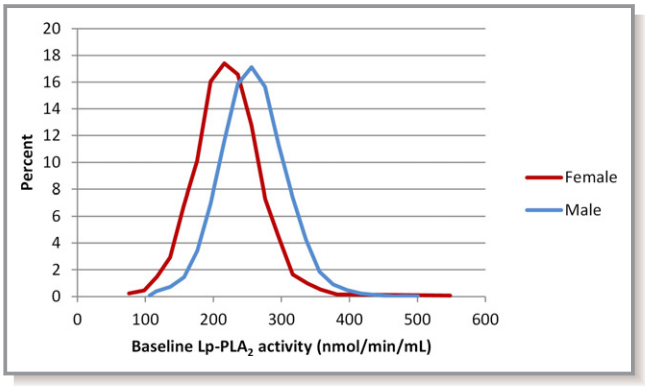

Figure 2. Distribution of lipoprotein-associated phospholipase A2 (Lp-PLA$_2$) activity levels. Baseline Lp-PLA$_2$ activity by sex, $P<0.0001$.

the estimated decrease in CHD events by pravastatin was reduced to 13%, accounting for ≈44% of the treatment effect. After adjustment for change in Lp-PLA$_2$ activity, the estimated decrease in CHD events by pravastatin was reduced to 10%, with 59% of the treatment effect accounted for by change in Lp-PLA$_2$ activity.

When the expanded endpoint of total CVD events was used, the relative risk reduction with pravastatin was reduced after adjustment for both changes in Lp-PLA$_2$ and LDL-C, to 0% (P=0.98). When these same analyses were conducted using quartiles of change, only change in Lp-PLA$_2$ activity, and not change in LDL-C, accounted for the pravastatin treatment effect.

Discussion

This study is the largest and the longest study of CHD patients to evaluate the relationship of Lp-PLA$_2$ activity to CHD outcomes. Baseline Lp-PLA$_2$ activity levels were not related to outcomes except for CHD death. Lp-PLA$_2$ activity levels at randomization were reduced after 1 year on pravastatin by 16%. Similar relative effects of pravastatin were observed within each subgroup defined by baseline Lp-PLA$_2$ quartiles but with greater absolute benefit among those with higher baseline levels. While baseline Lp-PLA$_2$ activity was not independently associated with outcomes after adjustment for all other risk factors, reduction in Lp-PLA$_2$ activity from randomization to 1 year was a significant independent predictor of CHD events even after adjustment for treatment and 23 baseline risk factors. This remained highly significant even after adjustment for change in brain natriuretic peptide, tropinin I, and LDL-C.

The major novel finding of this study is that change in Lp-PLA$_2$ levels accounted for at least as much of the pravastatin treatment effect on reducing CHD death and MI as did LDL-C reduction. While the changes in Lp-PLA$_2$ and LDL-C are related, this observation suggests that statins could reduce

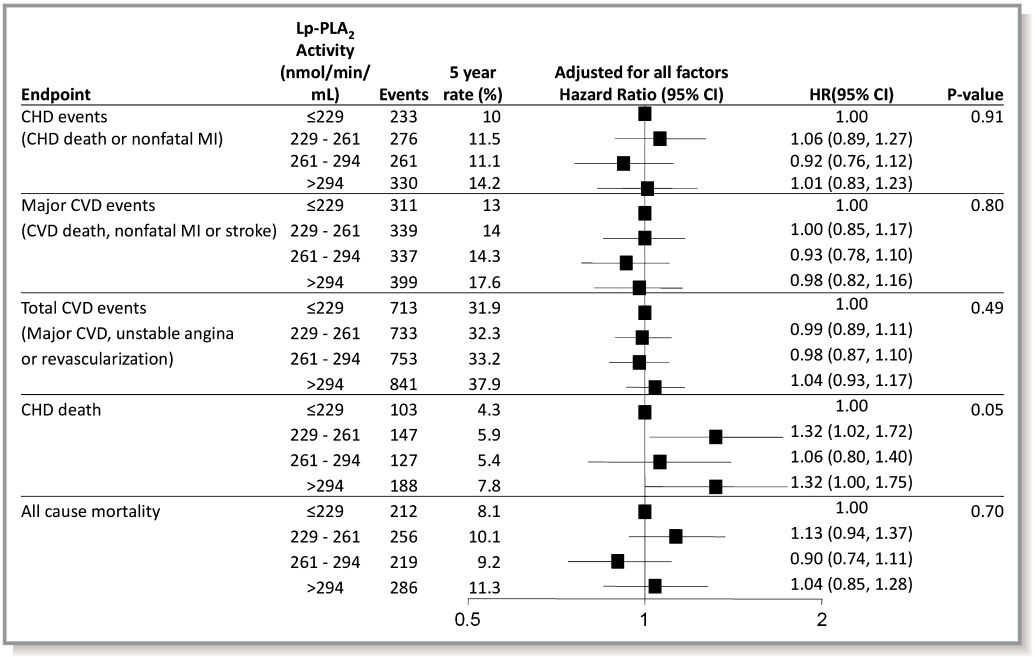

Figure 3. Baseline Lp-PLA$_2$ activity and prediction of CVD events. HR and 95% CI are adjusted for baseline variables: treatment, sex, stroke, diabetes, smoking, hypertension, total cholesterol, apolipoprotein B and A1, HDL-C, age, nature of prior ACS, timing of coronary revascularization, SBP, atrial fibrillation, eGFR, BMI, dyspnea class, angina grade, WBC, peripheral vascular disease, triglycerides, fasting glucose and aspirin at baseline. ACS indicates acute coronary syndromes; BMI, body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL-C, HDL cholesterol; HR, hazard ratio; Lp-PLA$_2$, lipoprotein-associated phospholipase A2; MI, myocardial infarction; SBP, systolic blood pressure; WBC, white blood cell count.

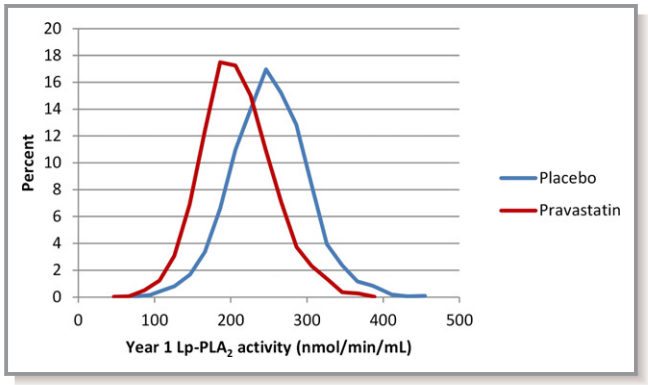

Figure 4. Distribution of lipoprotein-associated phospholipase A2 (Lp-PLA$_2$) activity levels. Lp-PLA$_2$ activity at 1 year by randomized treatment, $P<0.001$.

CHD risk in part by decreasing Lp-PLA$_2$ activity.[4,11] The CIs around estimates of proportion of treatment effect explained are wide and do not enable meaningful comparison between the estimates for LDL-C and Lp-PLA$_2$, which limits the conclusions that can be made from these results. However, all statistical models showed that change in Lp-PLA$_2$ was at least as strongly associated with CHD events as was change in LDL-C.

Lp-PLA$_2$ is produced by blood-borne inflammatory cells (monocytes, macrophages, T cells, mast cells) and is predominantly carried by LDL.[12] Pathological studies in animals have shown increased staining for Lp-PLA$_2$ in thin cap fibroatheroma and ruptured vulnerable plaques.[2] Higher plasma levels of Lp-PLA$_2$ have also been shown to be associated with coronary artery endothelial dysfunction in humans,[13] and coronary production of Lp-PLA$_2$ is correlated with the degree of plaque as assessed by intravascular ultrasound.[14]

In an analysis of 79 000 patients from 32 studies, Lp-PLA$_2$ activity was shown to be higher in men than in women and to be positively associated with LDL-C and inversely correlated with HDL cholesterol.[4] Higher plasma levels of Lp-PLA$_2$ were associated with cardiovascular events, including mortality, MI, and stroke.

Statins have been shown to reduce Lp-PLA$_2$ activity levels.[6,7,15,16] In the PRavastatin OR atorVastatin Evaluation and Infection Therapy (PROVE-IT) study, treatment with atorvastatin 80 mg/d was associated with a 20% reduction in Lp-PLA$_2$ activity at 30 days ($P<0.001$), whereas Lp-PLA$_2$ rose 3.6% with pravastatin 40 mg/d ($P<0.001$).[7] There was no placebo control group in this study. It is possible that as Lp-PLA$_2$ levels fall after acute coronary syndromes, as part of an acute-phase reaction, that the effect of pravastatin on decreasing Lp-PLA$_2$ activity may have been obscured by levels increasing back toward the baseline levels that existed before the acute coronary syndrome. In a study of subjects free of

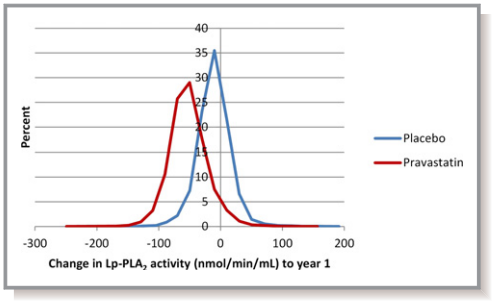

Figure 5. Distribution of lipoprotein-associated phospholipase A2 (Lp-PLA$_2$) activity levels. Change in Lp-PLA$_2$ activity from baseline to 1 year by randomized treatment, $P<0.001$.

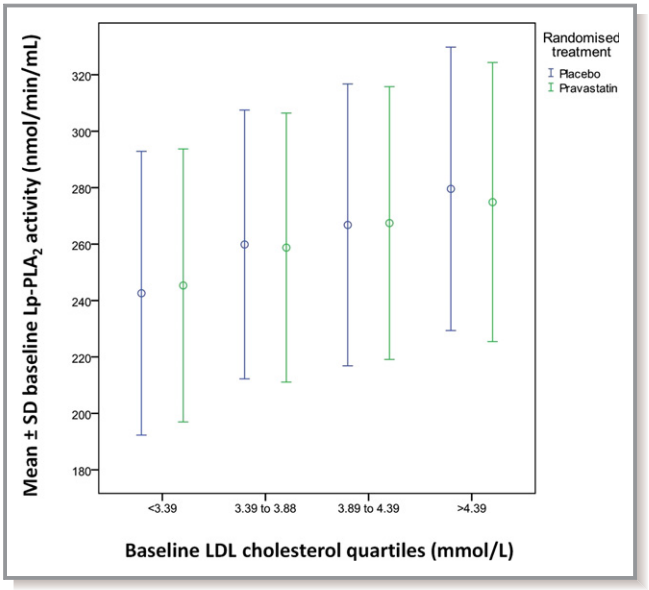

Figure 6. Lp-PLA$_2$ activity vs LDL-C at baseline. LDL-C indicates LDL cholesterol; Lp-PLA$_2$, lipoprotein-associated phospholipase A2.

CVD, Lp-PLA$_2$ levels were reduced 12.3% with pravastatin 40 mg at 12 months compared with placebo.[15]

In the Heart Protection Study (HPS), simvastatin reduced Lp-PLA$_2$ activity by 25%, but the effects of simvastatin on vascular outcomes did not vary according to Lp-PLA$_2$ levels.[6]

In the Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial, Lp-PLA$_2$ activity was associated with cardiovascular risk.[16] Rosuvastatin reduced Lp-PLA$_2$ activity by 33.2% ($P<0.0001$), and Lp-PLA$_2$ no longer predicted risk in patients treated with rosuvastatin. The mechanisms for inhibition of Lp-PLA$_2$ activity by statins are not well defined. However, simvastatin has been shown to reduce Lp-PLA$_2$ expression and activity in lipopolysaccharide-stimulated human myocyte-derived macrophages through inhibition of the mevalonate–geranylgeranyl pyrophosphate–RhoA–p38 mitogen-activated protein kinase pathway.[17]

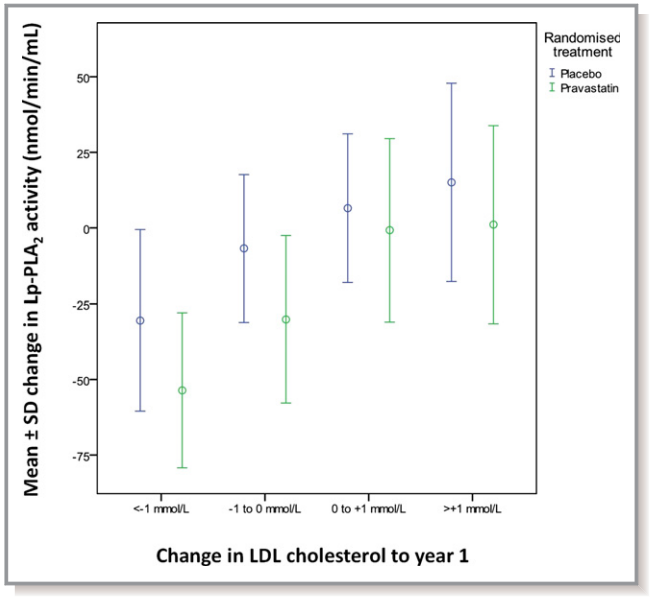

Figure 7. Change in Lp-PLA$_2$ activity vs change in LDL-C. LDL-C indicates LDL cholesterol; Lp-PLA$_2$, lipoprotein-associated phospholipase A2.

In the current study, it is not known if pravastatin also reduced the Lp-PLA$_2$ activity in plaques. If this occurred, the complexity of coronary lesions may have been reduced and potentially progression of the necrotic core may have been halted with stabilization of coronary atheromatous plaques. This could explain the reduction in CHD events that was observed. The results from the Cholesterol Treatment Trialists (CTT) overview, which did not use a landmark analysis, are consistent with most of the effects of statins being correlated with the size of the magnitude of reduction in LDL-C change, but this can still allow for additional mechanisms to contribute.[18] We have previously shown that ≈50%[9] of the treatment effect of pravastatin in the LIPID study was explained by reduction in LDL-C.

Darapladib, a potent reversible inhibitor of Lp-PLA$_2$, has been shown in diabetic, hypercholesterolemic pigs to reduce the inflammatory product of Lp-PLA$_2$ lysophosphatidylcholine content of coronary plaque and to reduce macrophage infiltration into the arterial wall and to reduce the necrotic core of plaque.[19] In humans, darapladib has been shown to lower Lp-PLA$_2$ activity by 60% and, in the Integrated Biomarker and Imaging Study-2 (IBIS 2) trial, to reduce the secondary end point of the necrotic core of coronary artery plaque.[20] Ongoing randomized trials of darapladib will establish whether lowering of Lp-PLA$_2$ activity may lower the risk of cardiovascular death, MI, and stroke after MI[21] and after an acute coronary syndrome.[22]

Limitations

There are several limitations of this study. These findings are from a clinical study and the randomized patients may not be

Table 3. Effect of Pravastatin Treatment on Clinical Events by Each Quartile of Baseline Lp-PLA$_2$ Activity

End Point	Lp-PLA$_2$ Activity (nmol/min per milliliter)	Placebo (5-y rate, %)	Pravastatin (5-y rate, %)	Hazard Ratio (95% CI)	NNT (Based on Common HR)	Interaction *P* Value Trend
CHD events (CHD death or nonfatal MI)	≤229	10.9	9.1	0.83 (0.64, 1.07)	45	0.36
	229 to 261	11.7	11.3	0.86 (0.68, 1.09	42	
	261 to 294	12.6	9.5	0.73 (0.57, 0.93)	39	
	>294	16.1	12.3	0.74 (0.59, 0.92)	31	
Major CVD events (CVD death, nonfatal MI, or stroke)	≤229	14.0	12.1	0.83 (0.66, 1.04)	35	0.27
	229 to 261	14.3	13.6	0.89 (0.72, 1.10)	34	
	261 to 294	16.9	11.6	0.66 (0.53, 0.83)	29	
	>294	19.7	15.5	0.75 (0.62, 0.91)	25	
Total CVD events (major CVD, unstable angina, or revascularization)	≤229	32.9	30.8	0.88 (0.76, 1.02)	25	0.63
	229 to 261	34.8	30.0	0.86 (0.74, 0.99)	24	
	261 to 294	35.4	31.0	0.83 (0.72, 0.96)	24	
	>294	40.3	35.5	0.84 (0.74, 0.97)	22	
CHD death	≤229	5.1	3.5	0.73 (0.49, 1.08)	87	0.84
	229 to 261	5.8	6.0	0.84 (0.60, 1.16)	77	
	261 to 294	6.4	4.3	0.68 (0.47, 0.96)	70	
	>294	8.4	7.2	0.81 (0.61, 1.08)	54	
All-cause mortality	≤229	9.1	7.2	0.78 (0.59, 1.02)	49	0.92
	229 to 261	10.4	9.8	0.81 (0.63, 1.03)	43	
	261 to 294	10.7	7.7	0.64 (0.49, 0.84)	42	
	>294	11.8	10.7	0.83 (0.65, 1.04)	38	

CHD indicates coronary heart disease; CVD, cardiovascular disease; HR, hazard ratio; Lp-PLA$_2$, lipoprotein-associated phospholipase A2; MI, myocardial infarction, NNT, number needed to treat.

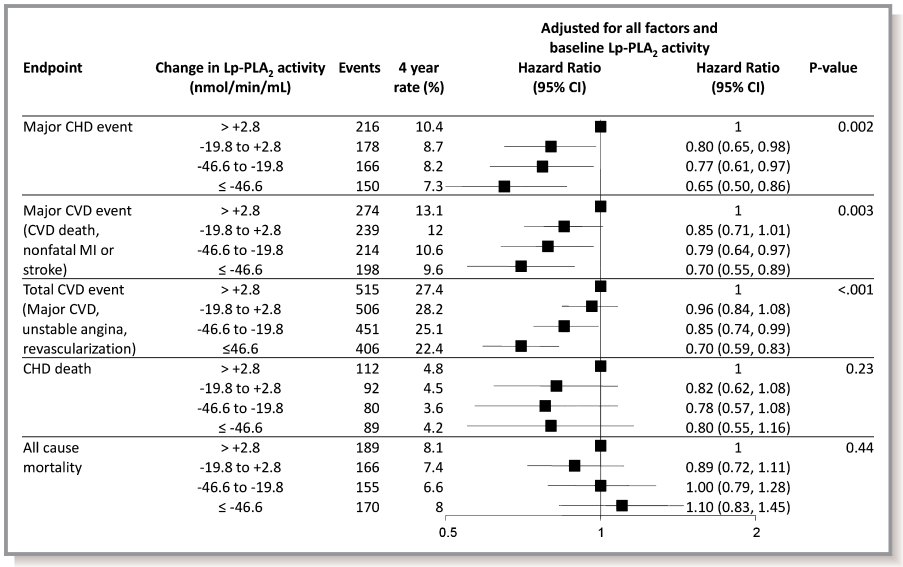

Figure 8. Quartiles of change in Lp-PLA$_2$ activity and prediction of CVD events. HR and 95% CI are adjusted for baseline variables: treatment, sex, stroke, diabetes, smoking, hypertension, total cholesterol, apolipoprotein B and A1, HDL-C, age, nature of prior ACS, timing of coronary revascularization, SBP, atrial fibrillation, eGFR, BMI, dyspnea class, angina grade, WBC, peripheral vascular disease, triglycerides, fasting glucose, aspirin at baseline and change in LDL. ACS indicates acute coronary syndromes; BMI, body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL-C, HDL cholesterol; HR, hazard ratio; Lp-PLA$_2$, lipoprotein-associated phospholipase A2; MI, myocardial infarction; SBP, systolic blood pressure; WBC, white blood cell count.

Table 4. Coronary Events After 1-Year with Adjustment for Baseline Factors, Baseline Biomarkers and Change in Biomarkers

Variables	Level	HR (95% CI)	*P* Value Trend
Baseline Lp-PLA$_2$ activity	≤229.142	1	0.04
	229.142 to 261.028	1.14 (0.91, 1.42)	
	261.028 to 293.767	1.11 (0.89, 1.39)	
	>293.767	1.27 (1.01, 1.58)	
Change in Lp-PLA$_2$ activity	≤−46.6057	1	<0.001
	−46.6057 to −19.8282	1.16 (0.92, 1.47)	
	−19.8282 to 2.8565	1.29 (0.99, 1.69)	
	>2.8565	1.61 (1.23, 2.11)	

Lp-PLA$_2$ indicates lipoprotein-associated phospholipase A2; HR, hazard ratio.
Baseline Factors: Treatment, sex, stroke, diabetes, smoking, hypertension, total cholesterol, apolipoprotein B and A1, HDL cholesterol, age, nature of prior acute coronary syndromes, timing of coronary revascularization, systolic blood pressure, atrial fibrillation, estimated glomerular filtration rate, body mass index, dyspnea class, angina grade, white blood cell count, peripheral vascular disease, triglycerides, fasting glucose, and aspirin at baseline.
Baseline Biomarkers: Baseline brain natriuretic peptide, baseline cystatin C, baseline D-dimer, baseline troponin I, and baseline Lp-PLA$_2$.
Change in Biomarkers: Change in brain natriuretic peptide, troponin I, and Lp-PLA$_2$ activity.

fully representative of patients seen in clinical practice. Biomarker data were not available in some patients. However, the patients excluded compared with the patients included were younger, more likely to be male, and more likely to not have a history of hypertension and to not have had coronary revascularization.

There are limitations in using a landmark analysis to determine what proportion of treatment effect can be explained by change in a particular biomarker. Measurement error in the landmark analyses would tend to underestimate the associations we found. Estimates of the proportion of treatment effect accounted for by change in a biomarker are inherently imprecise.[23] Nonetheless, the observation that changes in Lp-PLA$_2$ accounted for at least as much of the pravastatin effect as LDL-C reduction is informative and provides support for the hypothesis that part of the pravastatin effect is mediated through Lp-PLA$_2$ change.

Conclusion

Higher baseline levels of Lp-PLA$_2$ activity significantly predicted an increased risk of CVD events including CHD death and MI, total CVD events, and all-cause mortality, but not in multivariate analyses except for CHD death. Pravastatin significantly reduced Lp-PLA$_2$ activity over 1 year by 16% compared with placebo. Similar relative effects of pravastatin were observed within each subgroup defined by baseline Lp-PLA$_2$ quartiles and greater absolute benefit was found in those with higher baseline levels.

Reduction in Lp-PLA$_2$ strongly predicts a reduction in subsequent CHD events after adjustment for treatment, 23 baseline risk factors, and other biomarkers and for changes in other biomarkers and for reduction in LDL-C.

Changes in Lp-PLA$_2$ appears to account for much of the pravastatin treatment effect (more than half) and appear to be at least as important as changes in LDL-C. Some of the effect of Lp-PLA$_2$ change (and LDL-C change) may be surrogates for each other, but both appear to account for more of the treatment effect than change in LDL-C alone. This novel

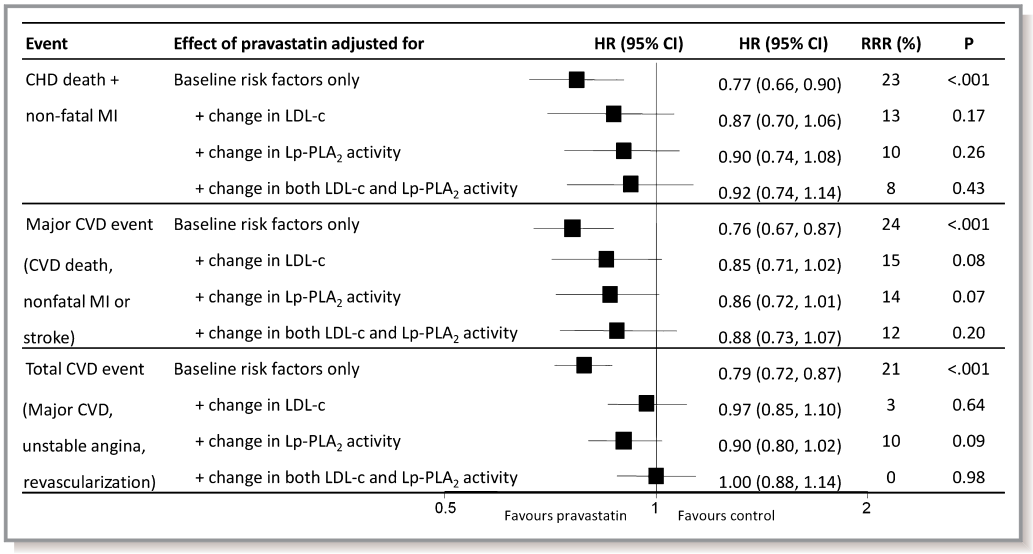

Figure 9. Effect of pravastatin on events after adjustment for changes in Lp-PLA$_2$ activity and LDL cholesterol (LDL-C).[9] Baseline and change in LDL-C and Lp-PLA$_2$ activity are expressed as continuous variables. CHD indicates coronary heart disease; CVD, cardiovascular disease; HR, hazard ratio; Lp-PLA$_2$, lipoprotein-associated phospholipase A2; MI, myocardial infarction; RRR, relative risk reduction.

finding requires validation in other studies, especially in ongoing trials. These results raise the possibility of additional mechanisms of statin effects on CVD event reduction and also provide a rationale for ongoing trials testing inhibitors of Lp-PLA$_2$ activity.

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Authors' Contributions

All authors had full access and contributed equally to the content of the manuscript. Dr Simes had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. The corresponding author has not been paid to write this article by a pharmaceutical company or any other agency.

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Disclosures

Dr White has received research grants from Sanofi Aventis; Eli Lilly; Medicines Company; NIH; Pfizer; Roche; Johnson & Johnson; Schering Plough; Merck Sharpe & Dohme; Astra Zeneca; GlaxoSmithKline; Daiichi Sankyo Pharma Development and Bristol-Myers Squibb and has served on Advisory boards for Merck Sharpe & Dohme, Roche, Astra Zeneca and Regado Biosciences. Drs Simes, Stewart, Barnes, Marschner, Thompson, Zeller, Colquhoun, Keech, and Hunt have no conflicts. Dr Blankenberg has received research grants from Boehringer Ingelheim, Bayer, Abbott Diagnostics, SIEMENS, and Thermo Fisher. He has received lecture fees from Astra Zeneca, Bayer, Boehringer Ingelheim, SIEMENS, Abbott Diagnostics and has been a member of Advisory Boards for Boehringer Ingelheim, Bayer, and ThermoFisher. Dr West has received research grants from Merck Sharpe & Dohme; Astra Zeneca; GlaxoSmithKline and Bristol-Myers Squibb. Dr Nestel is an Advisory Board member for AstraZeneca and Merck Sharpe & Dohme and does consultancy work for AstraZeneca and Merck Sharpe & Dohme. Dr Sullivan has received research grants from AstraZeneca, Eli Lilly, Merck Sharpe and Dohme, AMGEN and Sanofi Aventis and has served on advisory boards of Abbott, Merck Sharpe and Dohme and AMGEN. Dr Tonkin has received research grants from AstraZeneca; Bristol-Myers Squibb; Merck Sharpe & Dohme and NIH and has been a member of Advisory boards for AstraZeneca; Merck Sharpe & Dohme and Pfizer.

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