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Oxidized Phospholipids on Apolipoprotein B-100 and Recurrent Ischemic Events Following Stroke or Transient Ischemic Attack

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ABSTRACT

BACKGROUND Biomarkers to predict recurrent stroke and targets of therapy to prevent stroke are lacking.

OBJECTIVES This study evaluated whether patients with prior cerebrovascular events and elevated levels of oxidized phospholipids on apolipoprotein B-100 (OxPL-apoB), but without prior coronary artery disease (CAD), are at risk for recurrent stroke and CAD events following high-dose statin therapy.

METHODS In the SPARCL (Stroke Prevention by Aggressive Reduction in Cholesterol Levels) trial, OxPL-apoB levels were measured in 4,385 patients with stroke or transient ischemic attack at baseline and in 3,106 patients at 5 years following randomization to placebo or 80 mg atorvastatin. The primary endpoint was the time from randomization to a second nonfatal or fatal stroke. Secondary endpoints included first major coronary events and any cardiovascular event.

RESULTS Patients with recurrent stroke had higher baseline median OxPL-apoB levels than patients without (15.5 nmol/l vs. 11.6 nmol/l; $p < 0.0001$). After multivariable adjustment, elevated baseline OxPL-apoB predicted recurrent stroke (hazard ratio [HR]: 4.3; $p < 0.0001$), first major coronary events (HR: 4.0; $p < 0.0001$), and any cardiovascular event (HR: 4.4; $p < 0.0001$). These comparisons for any endpoint did not differ by treatment, shown as a nonsignificant interaction test. The net reclassification improvement, integrated discrimination improvement, and area under the receiver-operating characteristic curve (AUC) were all significantly improved by adding OxPL-apoB to the models, with ΔAUC +0.0505 ($p < 0.0001$) for recurrent stroke, ΔAUC +0.0409 ($p < 0.0001$) for first major coronary event, and ΔAUC +0.0791 ($p < 0.0001$) for any cardiovascular event.

CONCLUSIONS Elevated OxPL-apoB levels predicted recurrent stroke and first major coronary events in patients with prior stroke or transient ischemic attack. The lack of statin–OxPL-apoB treatment interaction suggested that OxPLs might be statin-independent therapeutic targets to reduce risk of cardiovascular events. (Lipitor in the Prevention of Stroke, for Patients Who Have Had a Previous Stroke [SPARCL]; NCT00147602) (J Am Coll Cardiol 2017;69:147–58) © 2017 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

apoB = apolipoprotein B-100

AUC = area under the receiver-operating characteristic curves

CVD = cardiovascular disease

LDL-C = low-density lipoprotein cholesterol

OSE = oxidation-specific epitope

OxPL = oxidized phospholipid

OxPL-apoB = oxidized phospholipids on apolipoprotein B-100

TIA = transient ischemic attack

Cerebrovascular disease is a major contributor to morbidity and mortality across the globe (1,2). In the United States, although the relative risk of dying from stroke is declining, approximately 800,000 people continue to experience new or recurrent strokes every year. As the population ages, cerebrovascular events have the potential to reach a higher incidence than myocardial infarction (MI). In fact, stroke burden is disproportionately higher in China, Africa, and South America compared with ischemic heart disease and with stroke rates in other counties (2). Although ischemic heart disease and ischemic stroke have commonalities in risk factors and underlying disease mechanisms, the strength of the association varies according to individual risk factors. Novel biomarkers and therapeutic targets would be useful to predict new or recurrent stroke and identify high-risk individuals for preventive measures.

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Oxidation-specific epitopes (OSE) are a major class of atherosclerosis-relevant antigens that define oxidative modifications on lipoproteins, apoptotic cells, and proteins (3). Present on apolipoproteins in the lipid phase as well as oxidized lipids covalently bound to amino acids on the protein moiety, OSEs share molecular identity with danger-associated molecular patterns on apoptotic cells as well as with pathogen-associated molecular patterns on microbial pathogens. OSEs are recognized by a common set of innate pattern recognition receptors, which have been evolutionarily selected to protect against the proinflammatory properties of OSEs (4,5). When OSEs activate arcs of immunity, the general response is to generate inflammation, which in turn mediates proatherogenic potential.

Phosphocholine-containing oxidized phospholipids (OxPLs) are well-studied OSEs that are highly immunogenic, proinflammatory, and present in atherosclerotic lesions of animals and humans, particularly in pathologically defined vulnerable and disrupted plaques (6,7). OxPLs are important contributors to early and late events in atherogenesis by activating proinflammatory genes in endothelial cells and macrophages (8), leading to inflammatory cascades in the vessel wall (9). Additionally, OxPLs on oxidized low-density lipoprotein cholesterol (LDL-C) lead to uptake of this even more damaging form of LDL-C by macrophages, promoting foam cell formation (10). OxPL can be measured on apolipoprotein B-100 (apoB) lipoproteins (OxPL-apoB) in plasma using the E06 natural antibody that recognizes the phosphocholine head group of OxPL. Elevated OxPL-apoB levels correlate with the presence of anatomical cardiovascular disease (CVD), and predict new CVD events in community-based settings in people with and without prior CVD (11–16).

The relationship of these biomarkers to cardiovascular outcomes in patients with prior cerebrovascular events is not defined. The SPARCL (Stroke Prevention by Aggressive Reduction in Cholesterol Levels) trial demonstrated that 80 mg atorvastatin reduced the overall incidence of recurrent stroke and cardiovascular events in patients with recent stroke or transient ischemic attack (TIA) but without known coronary heart disease (17). The aim of this current study was to assess the predictive value of OxPL-apoB levels, the effect of atorvastatin therapy, and their relationship to recurrent stroke or first major coronary event.

METHODS

OxPL-apoB levels were obtained from patients enrolled in SPARCL (17), a randomized trial comparing placebo versus 80 mg atorvastatin for secondary prevention of recurrent cerebrovascular events. Participants had experienced a stroke or TIA 1 to 6 months before study entry, had LDL-C levels of 100 to 190 mg/dl (2.6 to 4.9 mmol/l), and had no known coronary heart disease, and they were randomized to double-blind treatment with 80 mg of atorvastatin/day or placebo. The SPARCL trial's primary outcome was time from randomization to a first nonfatal or fatal stroke, the same endpoint that was used in the current substudy. Secondary endpoints were major coronary events (death from cardiac causes, nonfatal MI, or resuscitation after cardiac arrest) and any cardiovascular event. The latter covered the major coronary events plus stroke or TIA, unstable angina, any coronary event (acute coronary event plus a coronary

Diego on oxidation-specific antibodies and biomarkers related to oxidized lipoproteins. Dr. Witztum is a consultant to Ionis Pharmaceuticals, Cymabay Pharmaceuticals, Intercept Pharmaceuticals, and Prometheus; and has public stock in Ionis Pharmaceuticals. Dr. Tsimikas currently holds a dual appointment at the University of California-San Diego and Ionis Pharmaceuticals. Dr. Yang has reported that she has no relationships relevant to the contents of this paper to disclose.

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TABLE 1 Patient Characteristics

	Main Study		Biomarker Substudy					
	By Treatment			By Treatment		By Recurrent Stroke Event		
	Atorvastatin (n = 2,365)	Placebo (n = 2,366)	All Patients (N = 4,385)	Atorvastatin (n = 2,191)	Placebo (n = 2,194)	With Event (n = 544)	Without Event (n = 3,841)	p Value*
Age, yrs	63.0 ± 0.2	62.5 ± 0.2	62.8 ± 0.2	63.0 ± 0.2	62.5 ± 0.2	65.8 ± 0.5	62.3 ± 0.2	<0.0001
Male	1,427 (60.3)	1,396 (59.0)	2,625 (59.9)	1,322 (60.3)	1,303 (59.4)	362 (66.5)	2,263 (58.9)	0.0007
Risk factor								
Current smoker	452 (19.1)	456 (19.3)	836 (19.1)	412 (18.8)	424 (19.3)	108 (19.9)	728 (19.0)	0.3799
Hypertension	1,476 (62.4)	1,452 (61.4)	2,703 (61.6)	1,359 (62.0)	1,344 (61.3)	357 (65.6)	2,346 (61.1)	0.0412
DM	395 (16.7)	399 (16.9)	737 (16.8)	363 (16.6)	374 (17.0)	135 (24.8)	602 (15.7)	<0.0001
Lipids, mg/dl								
LDL-C	132.7 ± 0.5	133.7 ± 0.5	133.3 ± 0.4	132.7 ± 0.5	133.9 ± 0.5	133.6 ± 1.1	133.2 ± 0.4	0.5856
Total cholesterol	211.4 ± 0.6	212.3 ± 0.6	211.8 ± 0.4	211.3 ± 0.6	212.2 ± 0.6	211.1 ± 1.2	211.9 ± 0.5	0.6249
Triglycerides	144.2 ± 1.9	143.2 ± 1.4	143.1 ± 1.2	143.5 ± 2.0	142.7 ± 1.5	145.2 ± 2.9	142.8 ± 1.4	0.1998
HDL-C	50.0 ± 0.3	50.0 ± 0.3	50.0 ± 0.2	50.1 ± 0.3	49.9 ± 0.3	48.4 ± 0.5	50.2 ± 0.2	0.0077
OxPL-apoB, nmol/l	–	–	12.2 (6.7–18.7)	12.0 (6.8–18.5)	12.4 (6.6–18.9)	15.5 (11.0–21.3)	11.6 (6.3–18.2)	<0.0001

Values are mean ± SD or median (interquartile range) for continuous variables or n (%) for categorical variables. *p value for patients in the biomarker subgroup who experienced an event versus those that did not.

DM = diabetes mellitus; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; OxPL-apoB = oxidized phospholipids on apolipoprotein B-100.

revascularization procedure, angina or ischemia requiring emergency hospitalization), revascularization procedure (coronary, carotid, or peripheral), or clinically significant peripheral vascular disease.

Of the 4,731 SPARCL patients, 4,385 had available blood samples at baseline and 3,106 at 5 years for OxPL-apoB analyses and were thus included in the current study. The University of California-San Diego Human Research Subjects Protection program approved this research protocol, and all SPARCL participants gave written informed consent for the biomarker studies.

MEASUREMENT OF OxPL-apoB LEVELS. A chemiluminescent immunoassay was used to measure OxPL-apoB levels. The assay used the murine monoclonal antibody E06 that recognizes the phosphocholine group on oxidized but not native phospholipids (details in Byun et al. [16] and references therein) and results reported in nanomoles (nmol/l). The OxPL-apoB measure reflects OxPL on all apoB-100-containing lipoproteins, but we have shown previously that it primarily reflects the biological activity and clinical risk of lipoprotein(a) (Lp[a]), the major lipoprotein carrier of OxPL in plasma, by virtue of carrying ~85% of OxPL on apoB-100-containing lipoproteins (3,16,18–20). OxPL-apoB levels were measured in 2 batches, and a set of high and low standards were included on each plate to ensure uniformity of individual plates and to minimize batch-to-batch and plate-to-plate variability. Data from all of the standard curves from all plates were averaged and used to derive nmol/l units across all samples. Lp(a) levels were not measured in these subjects.

STATISTICAL ANALYSIS. Patient demographics and disease characteristics at randomization were compared between treatment groups using a chi-square test for categorical variables and a Wilcoxon rank sum test for continuous variables. Similar comparisons were conducted between those who did and did not experience an event. Changes from baseline to year 5 in OxPL-apoB levels were tested with a Wilcoxon signed rank test and compared with a Wilcoxon rank sum test between treatment groups and patients with and without a clinical outcome. Spearman correlation was calculated to assess associations between baseline and year 5 OxPL-apoB, and between OxPL-apoB and lipid and other nonlipid biomarkers. Associations among baseline and on-treatment (at 5 years) OxPL-apoB levels and recurrent stroke, first major coronary events, and any cardiovascular event (as described in the preceding text) were assessed in a Cox proportional hazards model after adjustment for age, sex, type of qualifying event (previous stroke or TIA), time since the qualifying event, investigator region, and difference between treatment groups (for the overall population), using a time to cerebrovascular events endpoint as the dependent variable, for all patients and for patients within each treatment group. OxPL-apoB was ranked into quartiles and expressed as 3 dummy indicator variables using the first quartile as the control. Treatment by OxPL-apoB interaction was examined separately in the same model with the

TABLE 2 Association Between Baseline OxPL-apoB Levels and Events

	Quartile of Baseline OxPL-apoB			
	1	2	3	4
n	1,105	1,098	1081	1101
Recurrent stroke	53.0 (4.8)	122.0 (11.1)	182.0 (16.8)	187.0 (17.0)
Year 4 event rate	4.5	10.0	15.5	14.9
Model 1*	1.0	2.4 (1.7-3.3)	3.7 (2.7-5.0)	3.7 (2.7-5.0)
Model 2†	1.0	2.4 (1.7-3.3)	3.6 (2.7-4.9)	3.6 (2.6-3.9)
Model 3‡	1.0	2.4 (1.7-3.3)	3.6 (2.6-4.8)	3.6 (2.7-4.9)
Model 4§	1.0	2.5 (1.8-3.5)	4.0 (2.9-5.4)	4.3 (3.1-5.9)
Treatment interaction p value		0.03‖	NS	NS
Major coronary event	12.0 (1.1)	51.0 (4.6)	65.0 (6.0)	54.0 (4.9)
Year 4 event rate	0.9	4.0	4.6	3.9
Model 1*	1.0	4.2 (2.2-7.9)	5.4 (2.9-10.0)	4.3 (2.3-8.1)
Model 2†	1.0	4.2 (2.3-7.9)	5.3 (2.8-9.8)	4.1 (2.2-7.7)
Model 3‡	1.0	4.2 (2.2-7.9)	5.2 (2.8-9.7)	4.2 (2.2-7.9)
Model 4§	1.0	3.9 (2.0-7.3)	4.8 (2.5-9.0)	4.0 (2.1-7.6)
Treatment interaction p value		NS	NS	NS
Any cardiovascular event	109.0 (9.9)	240.0 (21.9)	380.0 (35.2)	410.0 (37.2)
Year 4 event rate	8.9	19.6	31.5	32.8
Model 1*	1.0	2.3 (1.9-2.9)	4.0 (3.3-5.0)	4.3 (3.5-5.3)
Model 2†	1.0	2.4 (1.9-3.0)	4.0 (3.2-5.0)	4.2 (3.4-5.2)
Model 3‡	1.0	2.4 (1.9-3.0)	4.0 (3.2-5.0)	4.3 (3.5-5.3)
Model 4§	1.0	2.3 (1.9-3.0)	4.0 (3.5-5.0)	4.4 (3.6-5.5)
Treatment interaction p value		0.007¶	NS	NS

Values are n, n (%), or HR (95% CI) versus quartile 1. *Model 1: unadjusted. †Model 2: adjusted for age and sex. ‡Model 3: adjusted for age, sex, hypertension, DM, HDL-C, and difference between treatment groups. §Model 4: adjusted for age, sex, hypertension, DM, HDL-C, type of qualifying event (stroke or TIA), investigator region, time since the qualifying event, and difference between treatment groups. The 4-year event rate refers to Kaplan-Meier estimate of event rate at end of year 4 (day 1,460). ‖Placebo group: HR: 1.8 (95% CI: 1.2 to 2.8); atorvastatin group: HR: 3.7 (95% CI: 2.2 to 6.2). ¶Placebo group: HR: 1.8 (95% CI: 1.3 to 2.4); atorvastatin group: HR: 3.4 (95% CI: 2.4 to 5.0).

CI = confidence interval; HR = hazard ratio; NS = not significant; TIA = transient ischemic attack; other abbreviations as in Table 1.

addition of the interaction terms. OxPL-apoB was further evaluated by calculating the associated incremental area under the receiver-operating characteristic curves (AUC) to predict recurrent event of stroke, when OxPL-apoB was added in the logistic regression model together with other factors that are associated with the event: age, sex, hypertension, diabetes, high-density lipoprotein cholesterol (HDL-C), and study treatment. The change in net reclassification improvement (NRI) and integrated discrimination improvement (IDI) with the addition of OxPL-apoB were also calculated.

RESULTS

Baseline characteristics from the main SPARCL study (n = 4,731) and the OxPL-apoB biomarker substudy (n = 4,385) at the time of randomization are shown in Table 1; in the biomarker substudy, 2,191 patients were treated with 80 mg atorvastatin and 2,194 with placebo. Of the substudy subjects, 544 had recurrent stroke and 182 had a major coronary event during the study. In the main study, the event rate for the primary endpoint was 576 (12.2%) versus 544 (12.4%) in the current analysis. Patients with events were older, more likely male, and characterized by a higher prevalence of hypertension and diabetes and lower HDL-C levels, but similar LDL-C levels.

At baseline, OxPL-apoB levels were not significantly different between the placebo and 80 mg atorvastatin groups. However, patients with recurrent stroke had higher levels of OxPL-apoB at baseline than those without events: median 15.5 nmol/l (interquartile range [IQR]: 11.0 to 21.3 nmol/l) versus 11.6 nmol/l (IQR: 6.3 to 18.2 nmol/l) ($p < 0.0001$) (Table 1). Baseline median levels of OxPL-apoB were higher in patients with diabetes versus those without (mean 13.1 nmol/l [IQR: 7.3 to 19.0 nmol/l] vs. 12.0 nmol/l [IQR: 6.6 to 18.6 nmol/l]; $p = 0.0389$) using the Wilcoxon rank sum test. Baseline renal function using the Cockcroft-Gault formula to calculate creatinine clearance showed no association with OxPL-apoB levels.

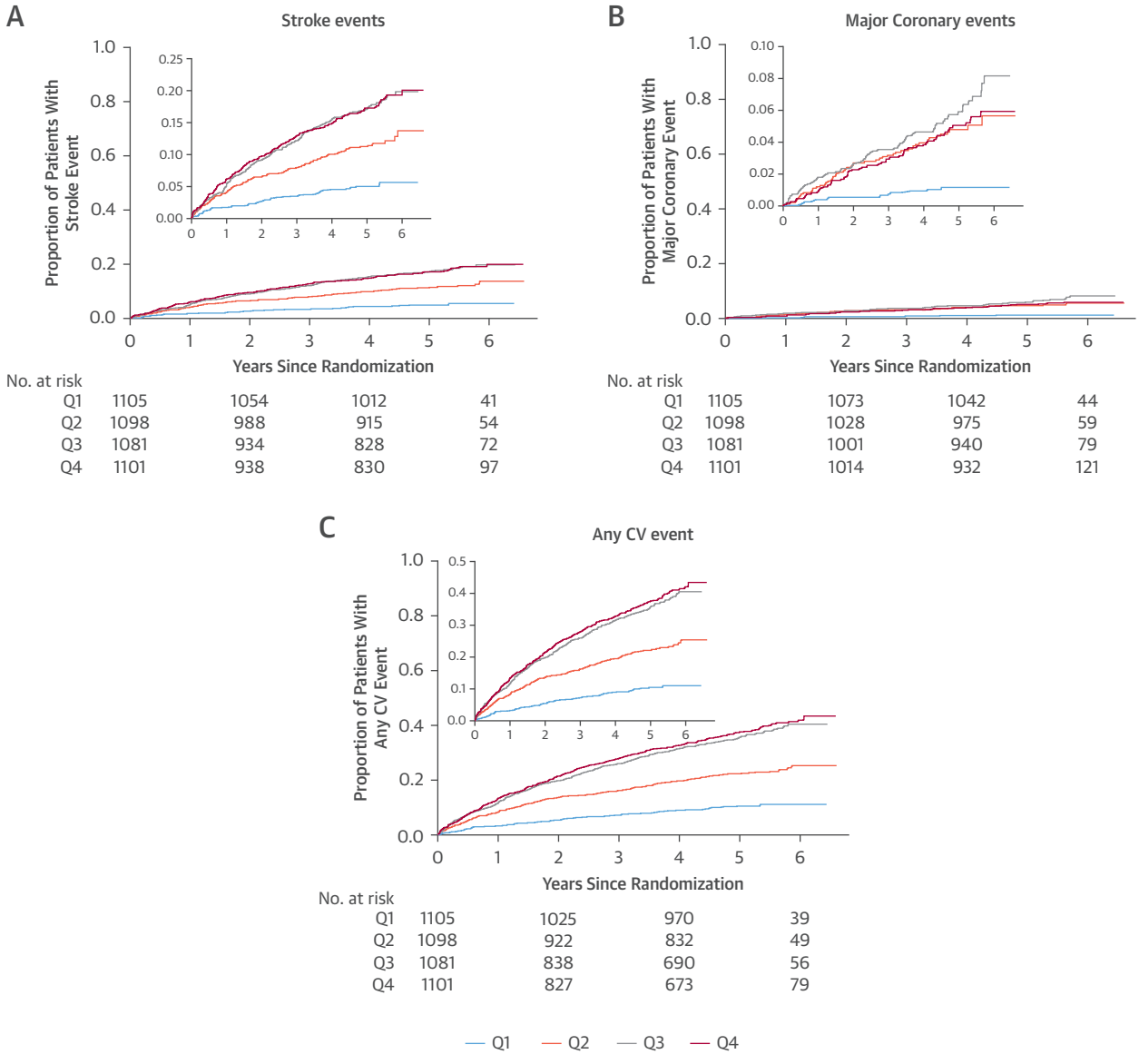
TABLE 3 Model 4: OxPL-apoB and Events

	Recurrent Stroke HR (95% CI)		First Major Coronary Event HR (95% CI)		Any Cardiovascular Event HR (95% CI)	
Quartile	Atorvastatin	Placebo	Atorvastatin	Placebo	Atorvastatin	Placebo
1	1.0	1.0	1.0	1.0	1.0	1.0
2	3.7 (2.2-6.2)	1.8 (1.2-2.8)	4.6 (1.6-13.6)	3.5 (1.6-7.7)	3.4 (2.4-5.0)	1.8 (1.3-2.4)
p value*	<0.0001	0.007	0.006	0.002	<0.0001	0.0001
3	5.0 (3.0-8.4)	3.4 (2.2-5.0)	6.7 (2.3-19.7)	3.8 (1.7-8.3)	5.0 (3.5-7.3)	3.5 (2.7-4.6)
p value*	<0.0001	<0.0001	0.0005	0.0009	<0.0001	<0.0001
4	5.2 (3.1-8.7)	3.8 (2.6-5.7)	4.0 (1.3-12.5)	4.1 (1.8-8.9)	5.5 (3.8-7.9)	4.0 (3.0-5.3)
p value*	<0.0001	<0.0001	0.015	0.0005	<0.0001	<0.0001

*p value vs. quartile 1.

Abbreviations as in Tables 1 and 2.

FIGURE 1 **Association Between Baseline OxPL-apoB Quartiles and Events**



The analysis of association between oxidized phospholipids on apolipoprotein B-100 (OxPL-apoB) and **(A)** recurrent stroke, **(B)** first major coronary event, and **(C)** any cardiovascular (CV) events was adjusted for age, sex, type of qualifying event (stroke or transient ischemic attack), investigator region, time since the qualifying event, and difference between treatment groups.

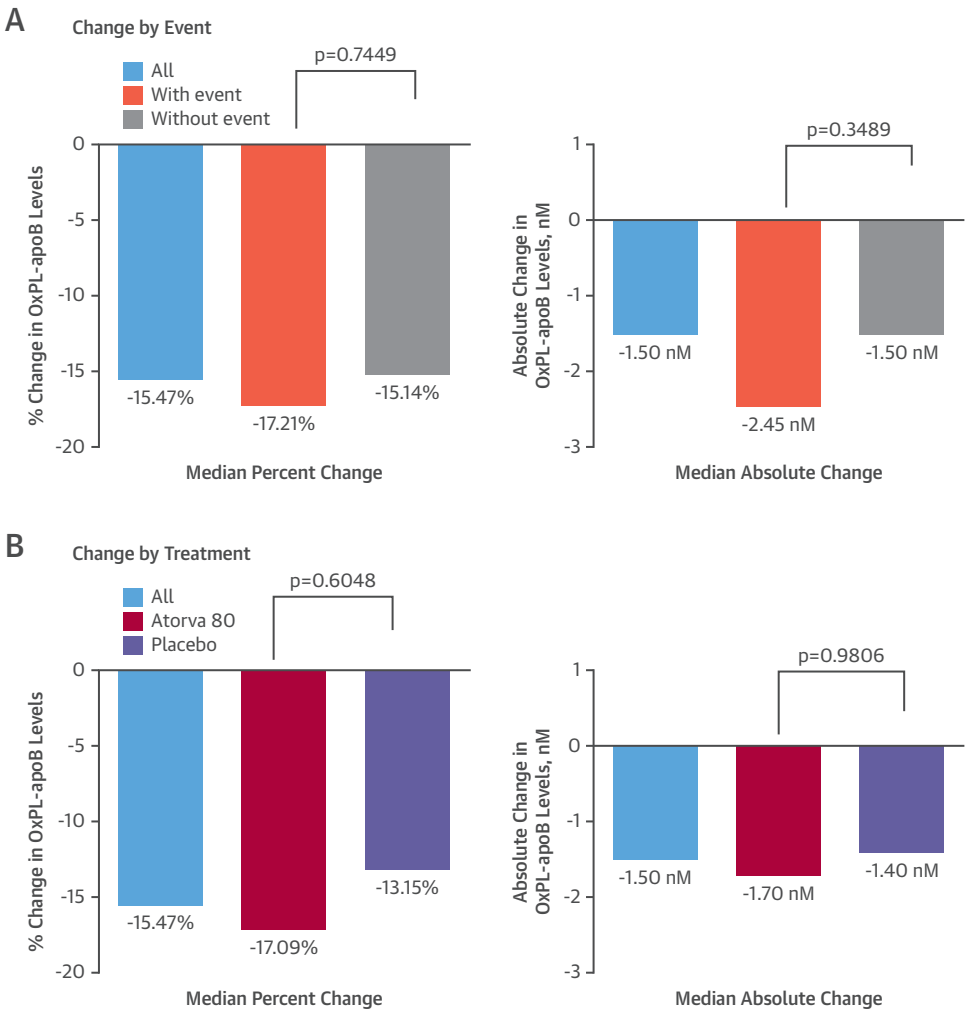
The association of OxPL-apoB with events was evaluated in 4 models:

- Model 1: unadjusted;
- Model 2: adjusted for age and sex;
- Model 3: adjusted for Model 2 variables plus hypertension, diabetes, HDL-C, and difference between treatment groups;
- Model 4: adjusted for Model 2 variables plus type of qualifying event (stroke or TIA), investigator region, time since the qualifying event, and difference between treatment groups.

Table 2 contains the analysis for all models, and Table 3 compares the atorvastatin and placebo groups for Model 4. All quartiles were associated with higher risk compared with quartile 1. Treatment by OxPL-apoB interaction (quartile 4 vs. 1) was not significant.

Kaplan-Meier curves on the basis of OxPL-apoB quartiles displayed a strong association among

FIGURE 2 5-Year Changes in OxPL-apoB Levels



There were no significant changes seen in oxidized phospholipids on apolipoprotein B-100 (OxPL-apoB) levels by **(A)** recurrent stroke or **(B)** treatment group from baseline to year 5.

baseline OxPL-apoB and recurrent stroke (Figure 1A), first major coronary event (Figure 1B), and any cardiovascular event (Figure 1C).

CHANGES ACCORDING TO EVENTS AND TREATMENT ASSIGNMENT. The median percent change in OxPL-apoB levels from baseline to year 5 was −17.2% (IQR: −47.3% to 35.7%; p = 0.2152) in patients with recurrent stroke and −15.1% (IQR: −50.8% to 50.0%; p = 0.2003) in those who did not experience an event; the difference between these patient groups was not statistically significant (p = 0.7449) (Figure 2A). Similar findings were observed for median absolute change in levels of OxPL-apoB (p = 0.3489). Additionally, similar data were present for percent change in OxPL-apoB and first major coronary event and any cardiovascular event (data not shown).

In the overall cohort, the median percent change in OxPL-apoB levels from baseline to year 5 was −15.5% (IQR: −50.4% to 48.7%) (Figure 2B). The median percent change was more apparent in the atorvastatin group (−17.1%; p = 0.3230) than in the placebo group (−13.2%; p = 0.2104), although the difference was not statistically significant (p = 0.6047) (Figure 2B). Similar findings were observed for median absolute change in levels of OxPL-apoB (p = 0.9806).

Receiver-operating characteristic curves for recurrent stroke were generated with 2 models for each

TABLE 4 Incremental AUC for Event Prediction

	All Patients*			Atorvastatin			Placebo		
Model	**AUC**	**ΔAUC**	**p Value**	**AUC**	**ΔAUC**	**p Value**	**AUC**	**ΔAUC**	**p Value**
Stroke									
1†	0.6284	–	–	0.6285	–	–	0.6357	–	–
2‡	0.6789	0.0505	<0.0001	0.6768	0.0483	0.0001	0.6866	0.0472	<0.0001
Major coronary events									
1†	0.6569	–	–	0.6473	–	–	0.6567	–	–
2‡	0.6978	0.0409	<0.0001	0.6859	0.0386	0.0067	0.7039	0.0483	0.0018
Any cardiovascular events									
1†	0.6285	–	–	0.6220	–	–	0.6278	–	–
2‡	0.7076	0.0791	<0.0001	0.6945	0.0725	<0.0001	0.7158	0.0880	<0.0001

*Analysis model also adjusted treatment effect when both treatment groups are included. LDL-C levels were matched. †Model 1 included age, sex, hypertension, DM, and HDL-C. ‡Model 2 added OxPL-apoB to model 1.

AUC = area under receiver-operating characteristic curve; other abbreviations as in Table 1.

of the endpoints: model 1 included age, sex, hypertension, diabetes mellitus, and HDL-C (LDL-C was matched); and model 2 further added OxPL-apoB. As seen in Table 4, adding OxPL-apoB significantly increased the AUC for prediction of recurrent stroke, major coronary events, and any cardiovascular events. Similar findings were present in the placebo and atorvastatin groups (Table 4).

NRI and IDI were determined for recurrent stroke, first major coronary event, and any cardiovascular event. In the total population and the atorvastatin and placebo groups, adding OxPL-apoB significantly changed both improvement indexes (Table 5).

BASELINE LDL-C AND PRESENCE OF DIABETES MELLITUS. We further analyzed the data by median baseline LDL-C (≤132 or >132 mg/dl) and median baseline OxPL-apoB (≤12.2 or >12.2 nmol/l). Higher levels of baseline OxPL-apoB were associated with increased risk of recurrent stroke ($p < 0.0001$), regardless of levels of LDL-C or diabetes. The OxPL-apoB level by LDL-C or by diabetes interaction was not significant. Diabetes ($p < 0.0001$) but not LDL-C ($p = 0.8812$) was associated with increased event rate of recurrent stroke (Table 6). Remarkably, subjects with diabetes and median baseline OxPL-apoB >12.2 nmol/l had a 47% rate of any cardiovascular events.

DISCUSSION

This study demonstrated that elevated baseline OxPL-apoB levels in patients with prior stroke or TIA, but no clinical evidence of coronary heart disease, predicted recurrent stroke, first major coronary events, and any cardiovascular events over a 5-year period. Several indexes of risk reclassification after adding OxPL-apoB to established risk factors showed enhanced predictive capability. The data further demonstrated that the risk mediated by elevated OxPL-apoB was not attenuated by high-dose statin therapy, despite an overall benefit otherwise, suggesting that the benefit of atorvastatin in reducing recurrent stroke is likely mediated through non-OxPL pathways. It also suggested that OxPLs may represent novel targets in reducing the risk of recurrent stroke and cardiovascular events. The Central Illustration displays a conceptual rendition of the effect of OxPL-apoB on recurrent stroke in the setting of statin or no statin therapy.

The current study supported the clinical utility of OxPL-apoB as a biomarker for the prediction of recurrent fatal or nonfatal stroke, as well as first major coronary events, in a group of subjects with exceedingly high CVD risk as reflected by the 29% 5-year total cardiovascular event rate. Patients enrolled in SPARCL had a history of ischemic stroke

TABLE 5 Change in NRI and IDI With OxPL-apoB Addition

	All Patients*		Atorvastatin		Placebo	
Model	**NRI**	**IDI**	**NRI**	**IDI**	**NRI**	**IDI**
Recurrent stroke	41.9	106.9	34.7	105.1	45.1	101.5
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
First major coronary event	31.6	49.7	30.4	47.9	36.3	54.6
p value	<0.0001	<0.0001	0.0101	0.0003	0.0002	<0.0001
Any cardiovascular event	54.0	162.7	47.6	174.4	61.7	188.1
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*Baseline variables include age, sex, hypertension, DM, and HDL-C. LDL-C levels were matched.

IDI = integrated discrimination improvement; NRI = net reclassification improvement; other abbreviations as in Table 1.

TABLE 6 Cardiovascular Event Risk

	OxPL-apoB ≤12.2 nmol/l		OxPL-apoB >12.2 nmol/l	
	LDL-C ≤132 mg/dl	LDL-C >132 mg/dl	LDL-C ≤132 mg/dl	LDL-C >132 mg/dl
Stroke	7.80	8.11	16.92	16.90
Major coronary event	2.81	2.83	4.80	6.13
Any cardiovascular event	15.23	16.41	35.20	37.23
	Non-DM	**DM**	**Non-DM**	**DM**
Stroke	7.35	11.2	15.26	24.31
Major coronary event	2.68	3.85	4.8	8.27
Any cardiovascular event	14.96	20.71	33.76	47.12

Values are %.
Abbreviations as in Table 1.

(~70%) or TIA (~30%); they tended to be young (mean age: ~63 years), active or former smokers (~60%), and hypertensive; they were almost uniformly on antiplatelet therapy, with a mean LDL-C ~133 mg/dl; and only ~2.5% were receiving statin therapy prior to randomization. The fact that OxPL-apoB predicted recurrent fatal or nonfatal stroke in this group of patients without coronary artery disease (CAD) potentially reflects the proinflammatory properties of OxPL in mediating ischemic cardiovascular events in multiple vascular territories. This is exemplified by recent findings that patients with elevated OxPL-apoB levels have augmented proinflammatory responses manifested by a variety of phenotypes of activated monocytes that are attenuated by inactivating OxPLs (18). OxPLs are also carried by monocyte chemoattractant protein-1 (21) and may enhance uptake of monocytes into the vessel wall, activate inflammatory pathways in monocytes, and upregulate interleukin-8 secretion (22).

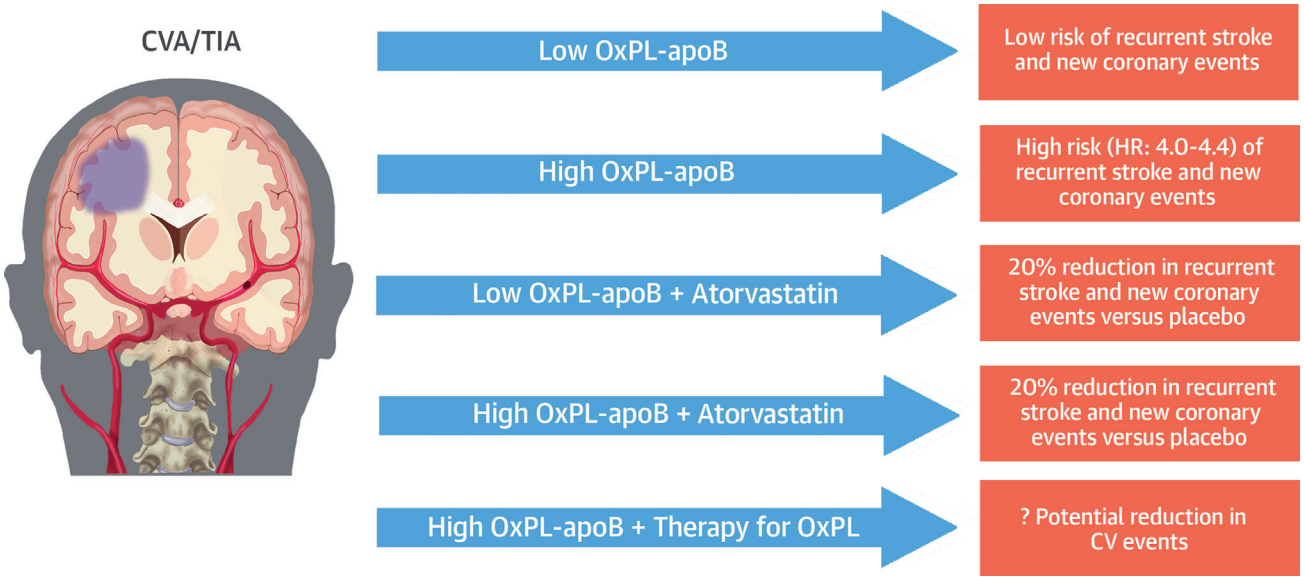
In the 15-year, prospective Bruneck study evaluating first-time events in the general community, OxPL-apoB was associated with time to first stroke, with an HR of 3.6 comparing third versus first tertile levels (14). That study was different yet complimentary to SPARCL in that Bruneck enrolled community-based subjects who were at much lower risk, whereas SPARCL involved much higher-risk patients who already had an event. Irrespective of these differences, the studies are pathophysiologically consistent, showing that OxPL-apoB can predict both first and recurrent cerebrovascular events. Furthermore, although this study was not designed as an outcomes study, elevated OxPL-apoB predicted first coronary events, which had a lower frequency than recurrent stroke. These data were consistent with prior observations on the association of OxPL-apoB with CAD events (11,13–16).

The baseline and changes in AUC, as well as the IDI and NRI, were consistent with prior OxPL-apoB and Lp(a) data from subjects in the general community from the Bruneck study with prospective 15-year follow-up: baseline AUC was 0.664 using Framingham variables, which increased to 0.705 by adding OxPL-apoB (ΔAUC 0.041) (14). Similarly, in a separate analysis of the Bruneck study, the AUC for Lp(a) using Framingham variables was 0.758, which increased to 0.774 (ΔAUC 0.016) (19). In contrast, most high-sensitivity C-reactive protein (hsCRP) studies have shown no significant increases in AUC (23). In the ARIC (Atherosclerosis Risk In Communities) study representing the general population, the addition of hsCRP and lipoprotein-associated phospholipase A2 to the traditional model increased the AUC from 0.0732 to 0.0774 (ΔAUC 0.042) (24). Evaluation of AUCs in patients with prior stroke has not been carried out as best as we could determine, which added to the uniqueness of this dataset from SPARCL.

A second interesting observation in SPARCL was that high-dose atorvastatin, despite significantly reducing recurrent cerebrovascular events by ~20%, did not seem to mitigate the risk of recurrent stroke associated with elevated baseline OxPL-apoB. Potential mechanisms of the salutatory effect of atorvastatin in reducing the risk of stroke cannot be determined from this particular study design. However, prior data have suggested that a combination of potential effects may be at play, including reducing plasma levels of atherogenic lipoproteins that limit the substrate for oxidation, potent antioxidant effects of atorvastatin metabolites, immunomodulatory effects, nitric oxide preservation, and beneficial effects on platelets and thrombosis pathways (25–27). The SPARCL findings suggested that statins do not have direct salutary effects on the OxPL-apoB pathway, which primarily reflects Lp(a)-mediated risk (3,18,20).

One potential explanation in understanding the lack of treatment effect of atorvastatin in reducing OxPL-mediated risk may be reflected in the unique Lp(a)-OxPL-statin relationship, on which we have previously reported, as discussed below. In several experimental and clinical studies, 3 key observations on the Lp(a)-OxPL relationship have been made. First, Lp(a), as opposed to LDL, is the main lipoprotein carrier of proinflammatory OxPL, carrying ~85 of OxPLs present on all lipoproteins (3,20). The ability of Lp(a) to bind OxPL appears to be unique to humans compared with other species with circulating Lp(a); it is mediated by unique species-specific differences in the lysine-binding pocket of KIV_{10} of apo(a), which

CENTRAL ILLUSTRATION **Oxidized Phospholipids as Predictors of Recurrent Stroke and First Coronary Events**



Byun, Y.S. et al. J Am Coll Cardiol. 2017;69(2):147–58.

In patients with brain injury from a prior cerebrovascular accident (CVA) or transient ischemic attack (TIA), various pathways may await **(arrows)** depending on baseline oxidized phospholipids on apolipoprotein B-100 (OxPL-apoB) levels, statin therapy, and the possibility of inactivating OxPL to potentially reduce further events on background statin therapy. CV = cardiovascular; HR = hazard ratio.

influences OxPL binding in adjacent areas of apo(a) (3). Furthermore, Lp(a) appears to be a specific carrier of OxPL and does not generally contain significant amounts of other OSEs, suggesting that the lipids on Lp(a) are not oxidized diffusely per se (20), but that Lp(a) acts as a carrier to bind and transport OxPL derived from cells and inflammatory sites containing OxPL (6,7). Second, much of the biological risk of Lp(a), as evaluated in epidemiological and clinical outcomes trials, seems to be related to its content of OxPL (11,13-16,28). For example, in all studies evaluated to date, OxPL-apoB is either a similar or superior predictor of clinical risk than Lp(a). Finally, all statins studied to date in measuring both Lp(a) and OxPL-apoB in the same datasets, including atorvastatin, rosuvastatin, pravastatin, and simvastatin/ezetimibe, tend to raise Lp(a) 10% to 20% and OxPL-apoB ~20% to 25% over relatively short-term periods of months to 1 year, as shown by a recent meta-analysis study in 3,870 patients (29). Because OxPLs have direct proinflammatory effects on cells of the vessel wall (8,9), either when present on Lp(a) or when generated locally from other lipoproteins or apoptotic cells, this increase in plasma Lp(a) and/or OxPL-apoB would potentially reduce the overall benefit of statins. Because OxPL-apoB has not been measured in most large outcome statin trials, one cannot currently evaluate this effect. In a meta-analysis by O'Donoghue et al. (30) in patients with established CAD, Lp(a) was a risk factor for subsequent events, an association that was most potent in patients with LDL-C ≥130 mg/dl (odds ratio: 1.46; 95% CI: 1.23 to 1.73; $p < 0.001$), whereas this relationship did not achieve statistical significance for studies with an average LDL-C <130 mg/dl (odds ratio: 1.20; 95% CI: 0.90 to 1.60; $p = 0.21$). However, in a more updated analysis (31) of Lp(a) in various landmark studies (32-35), elevated Lp(a) has remained a predictor of recurrent CVD events despite what are considered optimal and even supraoptimal (<70 mg/dl) LDL-C levels. This further suggests that some of the "residual risk" noted in statin trials may be due to persistently elevated Lp(a) and/or OxPL-apoB.

In SPARCL, the changes in OxPL-apoB from baseline to 5 years declined similarly in both the atorvastatin and placebo arms. Unfortunately, a sample in between these times was not available to assess whether there was a rise and fall in OxPL-apoB. One explanation for this is that the decline in OxPL-apoB levels in both groups may reflect the fact that OxPL-apoB is an

acute-phase reactant that has been shown to rise substantially post-acute coronary syndrome and remain elevated for 4 to 7 months afterward (7,36-39). Although this has not been shown previously for stroke or TIA, the fact that OxPL-apoB levels declined in the placebo group was consistent with a prolonged acute-phase response, as these patients were recruited within 1 to 6 months of the index event. A significant decline (−3.9%) in OxPL-apoB was also noted in the MIRACL (Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering) trial consisting of patients with acute coronary syndrome, but that study only had a 16-week follow-up. It is also possible that patients who had a robust rise in OxPL-apoB prior to being enrolled in SPARCL may have had OxPL-mediated risk that set them on a course of a recurrent event.

CLINICAL AND TRANSLATIONAL IMPLICATIONS. The lack of statin effect on OxPL-apoB risk was disappointing, but also suggested potentially novel biological pathways of stroke risk and opportunities to study whether targeting OxPL directly may reduce risk. We have previously proposed that OSEs can be targeted for "biotheranostic" clinical applications (i.e., biomarkers, therapeutics, and diagnostic molecular imaging) (40). For example, measuring OSEs, such as OxPL, in plasma would allow one to determine if a patient is at high risk for subsequent CVD events, as in SPARCL patients. If so, this patient could undergo an imaging procedure with either a nuclear or magnetic resonance contrast agent targeted to an OSE, such as an antibody or a mimotope (41), to localize the site where OSEs are present in significant amounts. In the case of stroke, imaging the carotid arteries, aortic arch, and intracranial vessels may reveal OxPL-rich plaques. Once identified, one can then prescribe an OSE-targeted drug to inactivate the OxPL to minimize inflammation and reduce subsequent CVD risk. In prior studies, we demonstrated that human antibody IK17, which binds both OxPL and malondialdehyde epitopes, reduced progression of atherosclerosis in murine models (10) as well as oxidized lipid deposits in transgenic zebrafish (42). In the case of prior stroke or TIA, one can test the hypothesis that targeting OxPL, in conjunction with high-dose statin therapy, will further reduce risk and disability of stroke and TIA in an event-powered outcomes trial. The achievement of very low LDL-C levels with proprotein convertase subtilisin/kexin type 9 inhibitors, which would not necessarily be used in most patients in SPARCL because the median baseline LDL-C was only 133 mg/dl absent statin therapy, may attenuate the risk of targeting OxPL, but this would need to be tested in clinical trials.

STUDY LIMITATIONS. Samples from intermediate time points were not available to assess temporal changes in OxPL-apoB levels and link these to outcomes. However, in prior studies, neither OxPL-apoB nor Lp(a) has correlated with levels of hsCRP or other inflammatory biomarkers, such as interleukin-6 or P-selectin, suggesting that they reflect different pathophysiological pathways (43,44). Additionally, Lp(a) levels were not available to assess the relationship to OxPL-apoB in this study and its role in recurrent stroke.

CONCLUSIONS

Elevated OxPL-apoB levels predicted recurrent stroke and first major coronary events in patients with prior stroke/TIA. Elevated OxPL-apoB levels are an independent and clinically informative biomarker in predicting cerebrovascular events in secondary-care prevention settings.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Patients with ischemic stroke face elevated risks of recurrent cerebral or de novo coronary events despite high-intensity statin therapy, and elevated blood levels of OxPL-apoB are predictive of these events.

TRANSLATIONAL OUTLOOK: Prospective studies should target pathways involved in the generation, transport, or inactivation of oxidative phospholipids as potential therapeutic strategies to reduce the risk of future ischemic events in statin-treated patients who are recovering from stroke or TIA.

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