

# Small apolipoprotein(a) isoforms may predict primary patency following peripheral arterial revascularization



Marianna Pavlyha, MD,<sup>a</sup> Madeleine Hunter, MD,<sup>b</sup> Roman Nowygrod, MD,<sup>b</sup> Virenda Patel, MD, MPH,<sup>b</sup> Nicholas Morrissey, MD,<sup>b</sup> Danielle Bajakian, MD,<sup>b</sup> Yihao Li, MS,<sup>a</sup> and Gissette Reyes-Soffer, MD,<sup>a</sup> *New York, NY*

## ABSTRACT

**Background:** High lipoprotein (a) [Lp(a)] is associated with adverse limb events in patients undergoing lower extremity revascularization. Lp(a) levels are genetically pre-determined, with *LPA* gene encoding for two apolipoprotein (a) [apo(a)] isoforms. Isoform size variations are driven by the number of kringle IV type 2 (KIV-2) repeats. Lp(a) levels are inversely correlated with isoform size. In this study, we examined the role of Lp(a) levels, apo(a) size, and inflammatory markers with lower extremity revascularization outcomes.

**Methods:** Twenty-five subjects with chronic peripheral arterial disease (PAD) underwent open or endovascular lower extremity revascularization (mean age, 66.7 ± 9.7 years; Female = 12; Male = 13; Black = 8; Hispanic = 5; and White = 12). Pre- and postoperative medical history, self-reported symptoms, ankle-brachial indices (ABIs), and lower extremity duplex ultrasounds were obtained. Plasma Lp(a), apoB100, lipid panel, and pro-inflammatory markers (IL-6, IL-18, hs-CRP, TNF $\alpha$ ) were assayed preoperatively. Isoform size was estimated using gel electrophoresis and weighted isoform size (*wIS*) calculated based on % isoform expression. Firth logistic regression was used to examine the relationship between Lp(a) levels and *wIS* with procedural outcomes: symptoms (better/worse), early primary patency at 2 to 4 weeks, ABIs, and reintervention within 3 to 6 months. We controlled for age, sex, history of diabetes, smoking, statin, antiplatelet, and anticoagulation use.

**Results:** Median plasma Lp(a) level was 108 (interquartile range, 44-301) nmol/L. The mean apoB100 level was 168.0 ± 65.8 mg/dL. These values were not statistically different among races. We found no association between Lp(a) levels and *wIS* with measured plasma pro-inflammatory markers. However, smaller apo(a) *wIS* was associated with occlusion of the treated lesion(s) in the postoperative period (odds ratio, 1.97; 95% confidence interval, 1.01-3.86;  $P < .05$ ). The relationship of smaller apo(a) *wIS* with reintervention was not as strong (odds ratio, 1.57; 95% confidence interval, 0.96-2.56;  $P = .07$ ). We observed no association between *wIS* with patient reported symptoms or change in ABIs.

**Conclusions:** In this small study, subjects with smaller apo(a) isoform size undergoing peripheral arterial revascularization were more likely to experience occlusion in the postoperative period and/or require reintervention. Larger cohort studies identifying the mechanism and validating these preliminary data are needed to improve understanding of long-term peripheral vascular outcomes. (JVS—Vascular Science 2024;5:100211.)

**Keywords:** Apo(a); Isoforms; Lipids; Lipoprotein(a); Peripheral arterial disease

Lipoprotein (a) [Lp(a)] is causal for the development of atherosclerotic cardiovascular disease and atherothrombosis as shown in epidemiological, Mendelian

randomization, and genome-wide association studies.<sup>1</sup> Elevated Lp(a) levels are independently associated with incidence of major adverse cardiovascular events (MACE) and major adverse limb events (MALE) after lower extremity endovascular<sup>2</sup> or open surgery<sup>3</sup> while controlling for other risk factors. Despite this, measurement of Lp(a) levels is not standard of care in those undergoing vascular intervention. Lp(a) particles contain two main apolipoproteins: B100 (apoB100) and apolipoprotein(a) [apo(a)]. Individuals can express one or two apo(a) isoforms and they vary in size depending on the number of Kringle-IV type 2 (KIV-2) repeats that are encoded by the *LPA* gene. Plasma Lp(a) levels and apo(a) isoform size are inversely correlated, with smaller isoforms generally dominating.<sup>1</sup> There are well-described mechanisms linking high levels of Lp(a) and atherosclerosis, including complement activation, inflammatory, and coagulation pathways.<sup>1</sup> Apo(a) can potentiate atherothrombosis through presence of lysine binding sites that allow accumulation of lipid in

From the Department of Medicine, Division of Preventive Medicine and Nutrition, Columbia University Vagelos College of Physicians and Surgeons<sup>a</sup>, and the Department of Surgery, Division of Vascular Surgery, Columbia University Vagelos College of Physicians and Surgeons.<sup>b</sup>

Provided by T32HL007343 (M.P.), UL1TR001873 (G.R.S.), and partial funding for stipend (M.P.) and manuscript processing funds were supported by a donation from Robin Chemers Neustein to the Reyes-Soffer Laboratory. Y.L. supported by Genome Center of Alzheimer's Disease (HCAD), 5U54AG052427-07.

Correspondence: Gissette Reyes-Soffer MD, 630 West 168th St, P&S 10th Floor, Room 501, New York, NY 10032 (e-mail: [gr2104@cumc.columbia.edu](mailto:gr2104@cumc.columbia.edu)).

The editors and reviewers of this article have no relevant financial relationships to disclose per the Journal policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

2666-3503

Copyright © 2024 The Author(s). Published by Elsevier Inc. on behalf of the Society for Vascular Surgery. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

<https://doi.org/10.1016/j.jvssci.2024.100211>

the arterial wall and activation of inflammatory pathways, owing to its binding of oxidized phospholipids.<sup>4,5</sup> High Lp(a) levels have also been associated with enhanced expression of adhesion molecules in endothelial cells and pro-inflammatory interleukins in monocytes and macrophages.<sup>6</sup> Finally, apo(a) has evolved from the plasminogen gene through duplication and remodeling<sup>7</sup> and its homology with plasminogen allows it to compete for its binding site, inhibiting thrombolysis and indirectly promoting a prothrombotic state. These mechanisms are of interest in vascular patency.

Numerous studies show that high Lp(a) levels are associated with increased incidence of claudication, symptoms progression, re-stenosis after intervention, hospitalization, and limb amputation.<sup>8</sup> Those with high Lp(a) have a higher risk of combined PAD outcomes after adjusting for other traditional risk-factors. Few studies, however, have looked at the association between apo(a) isoform size and peripheral arterial surgical outcomes. Available data suggests that both increased Lp(a) levels and the presence of small apo(a) isoforms are associated with the increased incidence of symptomatic PAD.<sup>9,10</sup> Whereas small apo(a) isoforms have been shown to be independently associated with three-fold increase in risk of major adverse cardiovascular events after coronary artery bypass grafting at long-term follow-up,<sup>11</sup> the role of this Lp(a) component on lower limb outcomes has not yet been explored. In view of previous findings and needs in the field, we developed a study using available stored plasma samples and outcomes data on subjects with PAD who underwent lower extremity revascularization. We examined the relationships between apo(a) isoform size, early primary patency, and rate of reintervention. In addition, given apo(a)'s role in propagating the inflammatory cascade implicated in atherosclerotic plaque formation,<sup>12-14</sup> we also assessed the relationship of pro-inflammatory markers (interleukin [IL]-6, IL-18, high-sensitivity C-reactive protein [hs-CRP], tumor necrosis factor alpha [TNF $\alpha$ ]) with apo(a) size and surgical outcomes.

There are ongoing studies focused on lowering Lp(a) by targeting the apo(a) component in phase III of development,<sup>15</sup> highlighting the need to understand which populations are at an increased risk and would benefit from treatment. Our small study provides preliminary insights that can be used to design larger studies in patients with advanced PAD. These studies may assist with improving risk prediction, stratification, and medical optimization of surgical patients with high Lp(a).

## METHODS

We analyzed data from all 27 participants recruited from the Columbia University Irving Medical Center (CUIMC) Vascular Surgery Clinic into a prospective cohort study (Anthropometric and Biomarker Assay, and Lower Extremity Arterial Revascularization

## ARTICLE HIGHLIGHTS

- **Type of Research:** Single-center prospective cohort study
- **Key Findings:** Twenty-five subjects with symptomatic peripheral arterial disease underwent open or endovascular lower extremity revascularization. Smaller apolipoprotein (a) (apo(a)) isoforms were associated with occlusion of the treated lesion(s) within 2 to 4 weeks (odds ratio, 1.97; 95% confidence interval, 1.01-3.86;  $P < .05$ ), suggesting apo(a) isoform size as a predictor primary patency in the early period after lower extremity intervention.
- **Take Home Message:** Subjects with high lipoprotein (a) levels generally have smaller apo(a) isoform sizes. We find that, in this small cohort, patients undergoing peripheral arterial revascularization subjects with small isoforms are at an increased risk of treated vessel occlusion in the perioperative period.

[ABALEAR]) between June 2018 and March 2021 (Supplementary Fig 1, online only). The study protocol was approved by the CUIMC Institutional Review Board (AAAL7756). Inclusion criteria encompassed adult subjects with a clinical diagnosis of PAD, who were planned to undergo non-emergent revascularization (open or endovascular) of the affected lower extremity. Those with ischemic wounds, active infectious process, or cardiovascular intervention within 12 months of enrollment were excluded. Two of the participants who underwent only diagnostic angiogram were excluded from final analysis. The type of intervention was at the discretion of the treating provider.

**Recruitment/enrollment.** Subjects that met inclusion and exclusion criteria were contacted prior to their scheduled operation, and informed consent was obtained. Medical and social history were collected by a licensed health professional, including demographic information (age, sex, self-reported race and ethnicity [SRRE]), history of smoking, hypertension, diabetes, carotid artery disease (CAD), or dyslipidemia. Medication use (antihypertensives, statins, antiplatelet, direct oral anticoagulants) and symptoms of rest pain or claudication (ambulatory distance in city blocks) were recorded. A preoperative physical exam was performed, which included distal pulses, a lower extremity arterial duplex ultrasound (US), ankle-brachial index (ABI), and body mass index. On the day of the surgical procedure, arterial blood samples were collected using EDTA tubes; plasma was separated and stored at  $-80^{\circ}\text{C}$ . Success of the procedure was confirmed at the conclusion of each intervention with either angiography or handheld Doppler.

**Study follow-up (2-4 weeks and 3-6 months).** Participants were seen in the office for their postoperative visits by their respective providers. During these visits, physical exam (pulses, US, ABIs) to assess for early duplex derived primary patency and the trajectory of self-reported subjective symptoms were determined based on presence or resolution of rest pain and changes in walking distance. These were then categorized as 'improved,' 'worsened,' or 'stayed the same.' In cases of multiple vessels intervention, absent blood flow in the most proximal intervened vessel constituted occlusion. If multiple vessels below the popliteal artery trifurcation were intervened on, multiphasic in-line flow preserved in at least one of the vessels constituted patency. Palpable pulses over the graft and distally constituted patency. Two subjects did not have a complete US assessment at first follow-up and were excluded from sub-analysis. Change in ABI<sup>16</sup> measurements were categorized as improved from preoperative measurement vs not improved (worsened or the same). Of note, ABI measurements falsely elevated due to calcified non-compressible vessels, incomplete, or missing for at least one of the visits were excluded from the sub-analysis. Hence, the available ABI data is limited. Toe pressures were not available on this cohort. In addition to the above, the 3- to 6-month visit included records of any interval interventions on the ipsilateral extremity. Decision for reintervention was based on symptom severity, physical exam findings, and imaging (ie, US) results.

Plasma Lp(a), apoB100, lipids (triglycerides [TG], total cholesterol [C], high density lipoprotein [HDL]) were automatically measured on Integra400plus (Roche). Plasma LDL-C levels were calculated using the Friedewald formula (one subject had a TG level >400 mg/dL and was not reported). Plasma apoB100 levels were measured by a human enzyme-linked immunosorbent assay (ELISA) from Mabtech, Inc. Lp(a) plasma concentration were measured using the isoform-independent sandwich ELISA developed by the Northwest Lipid Metabolism and Diabetes Research Laboratory at Medpace, Inc. Apo(a) isoform size measurements were performed by the same laboratory using agarose gel electrophoresis. ChemiDoc MP Imaging System was used to determine the isoforms using in-house standards and relative expression of each isoform was reported. Weighted isoform size (*wIS*) was calculated using the % isoform expression (two isoform masses were multiplied by their relative expression). Pro-inflammatory markers IL-6 and IL-18 were measured using ELISA kits (D6050, R&D Systems) and (DY318-05, R&D Systems), respectively. hs-CRP and TNF $\alpha$  were measured on an Integra400plus (Roche) using standardized methods at the Irving Institute for Clinical and Translational Research Biomarkers laboratory at CUIMC.

**Statistical analysis.** All data were analyzed using standard R software (Version 4.2.2). Normally distributed

variables were summarized by means and standard deviations (SDs). Lp(a) levels and TGs were summarized by medians and interquartile ranges. The relationship between Lp(a) levels with *wIS* in the three SRRE groups was studied by analysis of variance. Racial differences in Lp(a) levels and *wIS* were assessed using the median test. Due to small samples size, an implementation based on the Firth logistic regression<sup>17</sup> was chosen to examine the relationship between *wIS* with procedural outcomes: change in symptoms, primary patency of treated lesion(s), change in ABIs, and reintervention within 3 to 6 months. Results were reported as odds ratios (ORs). Sex, age, smoking status, history of diabetes, and statin, antiplatelet, and direct oral anticoagulant use were considered as potential confounders according to the literature, and were included in the regression.<sup>18-21</sup> Hypertension and body mass index were not integrated into the model based on their role in published studies of surgical outcomes.<sup>22,23</sup> We did not control for history of CAD, as literature shows that most individuals with diagnosis of PAD have some degree of CAD and at least 60% have significant CAD.<sup>24</sup>

## RESULTS

**Baseline characteristics.** Subject demographics and lipid and lipoprotein levels are shown in Table 1. The study population included 12 females and 13 males with a mean age of  $66.7 \pm 9.7$  years. Our cohort was multi-ethnic with 8 Black, 5 Hispanic, and 12 White subjects, representative of the available population at our institution. Rutherford staging was used to classify study participants, and all were found to belong to either stage 3 or 4. Each subject underwent a planned one-stage procedure at the beginning of the study. Five patients underwent open and 20 underwent endovascular revascularizations. Majority of subjects underwent infrainguinal intervention ( $n = 23$ ), and six required reintervention within 3 to 6 months. Individuals were mildly overweight and lipid levels were consistent with a population being treated for dyslipidemia. Despite use of lipid lowering therapies, the mean apoB100 levels were  $168.0 \pm 65.8$  mg/dL. Median plasma Lp(a) level was 108 nmol/L (interquartile range, 44-301 nmol/L). Primary patency rates at 2 to 4 weeks and 3 to 6 months were 76% and 68%. Rate of reintervention measured at 3- to 6-month visit was 24%.

**Lp(a) levels and isoform size.** Previous reports show that high Lp(a) levels are associated with worse outcomes after surgical intervention. We found that most of the individuals in our cohort had high Lp(a) levels. The mean *wIS* was  $18.4 \pm 4.1$  and, as expected, there was an inverse relationship between Lp(a) levels and *wIS* ( $R^2 = 0.20$ ;  $P = .02$ ) (Fig 1). Most individuals express two apo(a) isoforms; however, in our small diverse cohort, we found that one-half ( $n = 12$ ) of the individuals

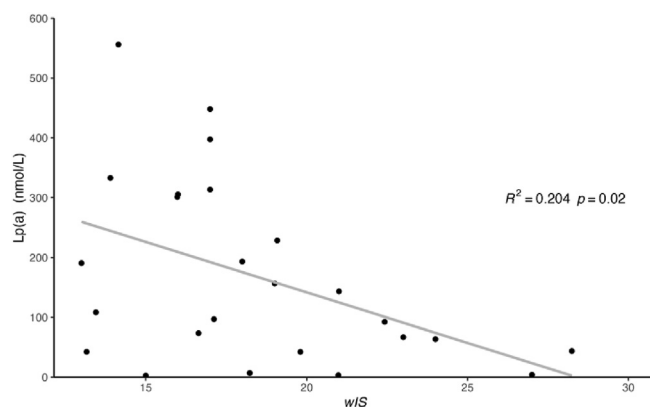
**Table I.** Baseline characteristics, lipid, and lipoprotein levels

Characteristic	Study sample		
Age, years	66.7 ± 9.7		
Range for age	43-79		
BMI, kg/m <sup>2</sup>	28.1 ± 5.3		
Race	Female (n = 12)	Male (n = 13)	
White	5	7	
Black	3	5	
Hispanic	4	1	
Clinical characteristics			
Diabetes mellitus	14 (56%)		
Hypertension	21 (84%)		
Hyperlipidemia	20 (80%)		
History of CAD	7 (28%)		
Prior coronary event	4 (16%)		
Ever smoker	20 (80%)		
Medical therapy			
Statin	19 (76%)		
Beta-blocker	12 (48%)		
DAPT or AP + DOAC	22 (88%)		
DOAC alone	1 (4%)		
AP alone	2 (8%)		
Total cholesterol, mg/dL	159 ± 46.1		
TG, mg/dL	148 (105-238)		
HDL-C, mg/dL	39.8 ± 14.0		
Calculated LDL-C, mg/dL <sup>a</sup>	78.7 ± 34.8		
Apo(a) wIS	18.4 ± 4.1		
	Black	Hispanic	White
Plasma Lp(a), nmol/L	68.3 (34.0-304.1)	228.6 (67.0-333.0)	125.9 (80.1-191.3)
Plasma apoB100, mg/dL	167.7 ± 45.1	225.6 ± 83.7	144.3 ± 59.2

AP, Antiplatelet; Apo(a), apolipoprotein (a); BMI, body mass index; CAD, coronary artery disease; DAPT, dual antiplatelet therapy; DOAC, direct oral anticoagulant; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); TG, triglyceride; wIS, weighted isoform size.  
Data are presented as number (%), mean ± standard deviation, or median (interquartile range).  
<sup>a</sup>Calculated LDL-C means reported for n = 24; one subject had >400 mg/dL TG levels (Supplementary Table, online only).

expressed only a single isoform (Supplementary Table, online only), with predominance of the small isoform (Fig 2).

**Relationship between Lp(a) levels and isoforms and surgical outcomes.** We did not find significant associations between Lp(a) levels and post-surgical symptoms, changes in ABIs, primary patency, or reintervention (Table II). Surgery type did not affect this result. However, wIS was significantly correlated with primary patency at 2 to 4 weeks (OR, 1.97; 95% confidence interval [CI], 1.01-

**Fig 1.** Negative relationship between lipoprotein(a) (Lp(a)) levels and weighted isoform size (wIS).

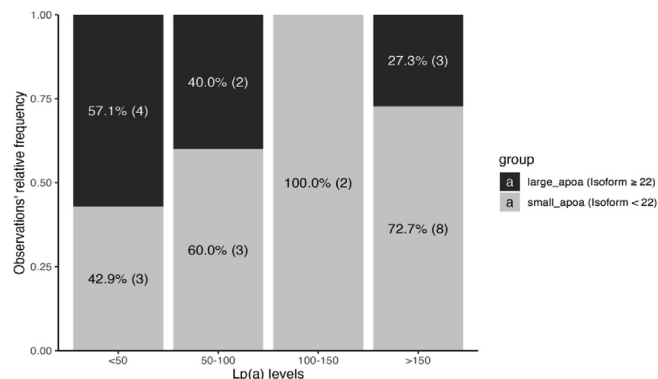
3.86;  $P < .05$ ) (Table III). Controlling for SRRE did not change these study outcomes. We observed a trend between wIS and reintervention in the 3- to 6-month period (OR, 1.57; 95% CI, 0.96-2.56;  $P = .07$ ). When we used predominant apo(a) isoform size as the primary variable in our model, and adjusted for plasma Lp(a) levels, our observed effect of apo(a) size on primary patency remained statistically significant (OR, 2.01; 95% CI, 1.03-3.93;  $P < .05$ ), suggesting that apo(a) was a stronger predictor of outcomes compared with Lp(a) levels in this cohort.

The type of surgical procedure could have affected our study outcomes; however, when we assessed the relationship between wIS isoform size and primary patency for subjects that only underwent endovascular intervention, we noted a similar trend (OR, 1.67; 95% CI, 0.95-2.94;  $P = .08$ ). We did not see an association between wIS and patient-reported symptoms or change in ABIs at 3 to 6 months. It is notable, however, that our sample size for analysis of ABIs was much smaller ( $n = 14$ ) due to the inability of all subjects to undergo the test reliably (ex, pain, non-compliant calcified vessels resulting in falsely elevated results) or missing measurement from some visits.

As expected, inflammatory markers IL-18 and hs-CRP were higher in this cohort when compared with published levels in healthy cohorts (Table IV). However, we found no significant relationship between plasma Lp(a) levels or wIS with IL-6, IL-18, hs-CRP, or TNF $\alpha$ . Additionally, these inflammatory markers were not associated with our surgical outcomes.

## DISCUSSION

High Lp(a) levels are associated with adverse limb events in patients with symptomatic PAD undergoing lower extremity revascularization.<sup>25-27</sup> International guidelines (Canadian<sup>27</sup> and ECC<sup>28</sup>) advocate for all individuals to have Lp(a) measured once in a lifetime. In the United States, the American Heart Association



**Fig 2.** Apolipoprotein (a) [Apo(a)] size distribution by lipoprotein(a) [Lp(a)] level quartiles (nmol/L). *Gray*: small apo(a) isoforms; *Black*: large apo(a) isoforms. Percentages and absolute numbers in parentheses represent the distribution of patients with small vs large apo(a) isoforms within each Lp(a) level quartile.

**Table II.** Effects of lipoprotein(a) (Lp(a)) on surgical outcomes

Outcome	Sample, n	Firth model	
		estimate (95% CI)	P value
Δ Symptoms	25	0.17 (−0.89 to 1.23)	.75
Primary patency	23	0.32 (−0.68 to 1.33)	.53
Δ ABI	14	0.92 (−1.57 to 3.41)	.47
Reintervention	25	0.13 (−0.89 to 1.15)	.80

ABI, Ankle-brachial index; CI, confidence interval. Weighted isoform size and lipoprotein(a) were standardized. Statistical significance =  $P < .05$ . Firth model: exponential estimate (95% CI). Available number of subject data for each outcome (n = X) are provided. Complete ABI data is missing in 11 subjects.

suggests using Lp(a) levels as a ‘risk enhancer.’<sup>29</sup> However, there are no specific guidelines for Lp(a) measurements in patients with advanced PAD that are referred for surgical intervention. All subjects in our study have been diagnosed with at least one metabolic disease; however, they were not screened for Lp(a) by their primary providers; and the majority were found to have elevated levels.

Although our study sample is small, which limits the generalizability of the results, it features a good representation based on gender and race. These data can be used to develop well-controlled cross-sectional studies that can examine the role of apo(a) in larger populations with PAD. Most individuals at risk or that have atherosclerotic cardiovascular disease, obtain laboratory measurement of low-density lipoprotein cholesterol (LDL-C), an apoB100-containing particle. Physicians rely on these measurements for prescribing lipid therapies, such as statins, in clinical practice. We observed that, in our cohort, 76% of individuals were prescribed statins. Although statins significantly lower LDL-C, they tend to increase Lp(a) by about 11%.<sup>1</sup> It is important to note,

**Table III.** Effects of apolipoprotein (a) (apo(a)) weighted isoform size (WIS) on surgical outcomes

Outcome	Sample, n	Firth model	
		estimate (95% CI)	P value
Δ Symptoms	25	0.25 (−1.01 to 1.50)	.70
Primary patency	23	−2.81 (−5.57 to −0.04)	<b>&lt;.05</b>
Δ ABI	14	0.30 (−2.43 to 3.01)	.84
Reintervention	25	−0.53 (−2.59 to 1.54)	<b>.07</b>

ABI, Ankle-brachial index; CI, confidence interval. Weighted isoform size and lipoprotein(a) were standardized. Statistical significance =  $P < .05$ . Firth model: exponential estimate (95% CI). Available number of subject data for each outcome (n = X) are provided. Complete ABI data is missing in 11 subjects. Boldface P values indicate statistical significance.

**Table IV.** Comparison of inflammatory marker levels in study subjects and healthy controls

Marker	PAD cohort	Healthy controls <sup>a</sup>
IL-6, pg/mL	3.3 ± 2.5	0.01-11.5 <sup>38</sup>
IL-18, pg/mL	274.2 ± 147.5	80-120 <sup>39</sup>
hs-CRP, mg/dL	3.6 ± 4.6	<0.3 <sup>40</sup>
TNFα, pg/mL	1.4 ± 0.7	0-2.2

CRP, C-reactive protein; IL, interleukin; PAD, peripheral arterial disease; TNF, tumor necrosis factor. Data are presented as mean ± standard deviation. Study cohort: n = 25. <sup>a</sup>Healthy controls from published literature.

however, that the laboratory levels of LDL-C in plasma are known to be confounded by the presence of Lp(a)-C,<sup>30</sup> which contains both apoB100 and apo(a). The reason for this overlap lies in similar plasma densities between Lp(a) and LDL particles. Thus, in patients with high levels of Lp(a), the actual LDL-C is lower than the reported value, and some investigators believe apoB100 to be a more accurate measure of cardiovascular risk compared with LDL-C.<sup>31</sup> This is important, because most vascular patients are prescribed statins, which work via increased LDL receptor expression, facilitating clearance of LDL-C, but not Lp(a). Patients that have elevated LDL-C while on optimal LDL lowering therapy would benefit from having their Lp(a) measured and treated, as there is a high probability that the increased cholesterol is carried by Lp(a) particles. We observe this in some patients in our population as well, and this may explain why some patients, despite LDL-C lowering by statins, remain at risk.<sup>32</sup> Two of 19 subjects in the study who were taking statins had above normal LDL-C levels; both had Lp(a) measurements >200 nmol/L. In this population where Lp(a) is high, this particle may carry most of the cholesterol.

Although there has been great interest in Lp(a) levels and surgical outcomes, few studies have looked at the role of apo(a) size in vascular surgery, and specifically, there are no existing reports on patency after lower

extremity revascularization. The *LPA* gene encodes for two apo(a) isoforms that comprise the Lp(a) particle. Variations in size of the apo(a) isoforms are driven by the number of KIV-2 repeats. Small number of KIV-2 repeats lead to small apo(a) size and are associated with higher Lp(a) levels, posing an increased risk for cardiovascular disease, including PAD.<sup>9</sup> Similar to other cohorts,<sup>1</sup> the current study finds an inverse association between plasma Lp(a) levels and apo(a) allele size, with smaller isoforms associated with higher Lp(a) levels.<sup>33</sup> Although ~20% of the general population is expected to have high Lp(a) and express two apo(a) isoforms,<sup>1</sup> close to one-half of our subjects with high median Lp(a) expressed only one isoform size (Supplementary Table, online only). Previous studies in individuals with PAD showed that both increased Lp(a) serum concentrations (75th percentile) and the small apo(a) phenotype are strongly associated with symptomatic PAD, independent of other confounders.<sup>9</sup> We did not see any significant correlation between Lp(a) levels and outcomes, but when controlled for Lp(a) levels, the predictive association of *wIS* with primary patency was statistically significant. Although data in the literature regarding disease predictability of Lp(a) vs apo(a) is conflicting, there is evidence that the strength of the association is higher for apo(a) isoform size in certain diseases.<sup>34</sup> To our knowledge, there are no reports assessing the correlation between apo(a) size and primary patency. Our preliminary study finds that small apo(a) size strongly correlates with primary patency within 2 to 4 weeks with a trend towards a higher rate or reintervention at 3 to 6 months. Although more comprehensive evaluation of the prognostic relationship between apo(a) size and outcomes of peripheral arterial interventions would be served in a randomized or cross-sectional study, these results support what is already known about apo(a)'s mode of action. We postulate that the probable mechanisms lie in the apo(a)'s kringle structure having homology with fibrinolytic proenzyme plasminogen, but inactive in fibrinolysis. Small apo(a) isoform size resulting from low *LPA* KIV-2 number of repeats can slow fibrinolysis and thus indirectly promote thrombosis, leading to increased risk of perioperative complications such as graft occlusion or thromboembolic events.<sup>35</sup> Additionally, apo(a) potentiates thrombosis by binding and inactivating tissue factor pathway inhibitor (TFPI).<sup>36</sup> These pro-thrombotic actions of the apo(a) could also explain the high rate of early reintervention in this cohort with high Lp(a), particularly as five of our six subjects in the group requiring reintervention had small apo(a) (Supplementary Fig 2, online only).

A study from a United Kingdom biobank studied approximately 460 thousand participants and showed significant differences in Lp(a) concentrations across racial subgroups, specifically with higher Lp(a) levels seen within the South Asian and Black populations

compared with Whites.<sup>37</sup> Although on average Hispanics had higher values than Whites in our cohort, there were no statistically significant differences in Lp(a) values and apo(a) size among different races, and race had no impact on most surgical outcomes. This could be due to our limited sample size.

Some of Lp(a)'s role in disease risk has been linked to inflammatory profiles. Specifically, elevated TNF- $\alpha$ , IL-6, and CRP levels<sup>12,38</sup> at baseline correlate with worse vascular outcomes in patients with diabetes, PAD, and chronic limb-threatening ischemia undergoing endovascular procedures.<sup>14</sup> We did not find any significant correlations with circulating inflammatory markers and Lp(a), *wIS*, or surgical outcomes; however, we did not measure oxidized phospholipids, which may add new information in patients with PAD as it has in other described populations.<sup>39</sup> Of note, it has also been reported that statins decrease the serum levels of hs-CRP<sup>12</sup> and downregulate IL-6.<sup>13</sup> Majority of our subject were taking high-dose statins (which also tend to increase Lp(a)), which may be contributing to our observations.

Although there are no currently available targeted therapies to treat high Lp(a), some available agents that lower apoB100 and LDL-C also decrease Lp(a) modestly, such as niacin, Ezetimibe, and PCSK9 inhibitors. The United States Food and Drug Administration has also recently approved lipoprotein apheresis for high-risk cases with Lp(a) >60 mg/dL and LDL-C >100 mg/dL and with either documented CAD or PAD.<sup>1</sup> The two apo(a) targeted therapies, antisense oligonucleotides and siRNA, are also in phase III studies and are expected to report their initial results in 2024. While we wait for this data, new studies now suggest the benefit of dual anti-platelet therapy (DAPT) in patients with high Lp(a).<sup>40</sup> Particularly, recent outcomes in these patients undergoing percutaneous coronary intervention, showed that prolonged DAPT (>1 year) reduced ischemic cardiovascular events, all-cause mortality, cardiac mortality, and definite/probable stent thrombosis, without increase in clinically relevant bleeding risk compared with  $\leq$ 1-year DAPT.<sup>41</sup> Data from this small study cohort raise the question whether we need to rethink risk assessment and postoperative management in patients with high Lp(a) and small apo(a) size undergoing lower extremity interventions. As the importance of Lp(a) becomes more recognized in clinical practice,<sup>37,42</sup> and we review cardiovascular outcome data from phase III apo(a) targeted treatments, developing larger studies that have more stringent control of patient selection and type of intervention would serve as the next step in validating the generalizability of our findings.

Understanding the impact of apo(a) isoform size on Lp(a) levels and surgical outcomes may guide risk stratification and therapeutic decision-making when it comes to preoperative optimization and medical management postoperatively. Lp(a) levels are stable throughout

lifetime. Although measuring apo(a) size may not be feasible in all patients and is currently not used in clinical practice, as genetic testing becomes more widely available, assessing KIV-2 size associated with the *LPA* gene may help identify individuals at higher risk of developing PAD and worse outcomes. Given Lp(a) genetic predisposition and low variability of levels throughout lifetime, one measurement may be sufficient and is the standard of care in many European countries.<sup>28</sup>

Further research in larger cohorts is needed to establish a more robust understanding of the mechanisms and impact of apo(a) isoform size on surgical outcomes in patients with PAD. Randomized clinical trials to explore potential medical therapy strategies to mitigate associated risks would aid in providing guidelines for perioperative management of these individuals.

**Study limitations.** Overall, this was a sample of convenience. Subject samples and prospectively collected data from ongoing biomarker study (ABALEAR) were available to our lipid team to examine additional affecters on surgical outcomes. Although subject baseline characteristics are comparable to larger cohorts, we acknowledge that sample size is small and significantly limit the generalizability of the results. We used Firth logistics regression to account for small sample size. Our cohort is heterogenous in terms of presenting symptoms with surgical interventions geared towards each patient's needs, but ultimately 'no leg is alike,' and the cohort represents a diverse spectrum of lower extremity disease and intervention seen in vascular surgery practice. We acknowledge that primary patency was measured in the early postoperative period at 2 to 4 weeks, during which patency may also be considered as a measure of procedure success or as a perioperative complication. Additionally, due to limited size, associations between peripheral interventions and Lp(a) levels/apo(a) size was done without correction for multiple testing.

## CONCLUSION

In this limited cohort, most subjects with chronic symptomatic PAD had elevated Lp(a) levels without being screened by their medical providers outside of the study, pointing to the need to increase awareness for Lp(a) screening in vascular patients. Small apo(a) isoform expression could be an important risk predictor not only for the development of PAD, which has already been established in literature, but also as a driver of worse short-term surgical outcomes. Further studies identifying the mechanism and relationship of these findings to long-term peripheral vascular outcomes are warranted, specifically identifying the role of apo(a) size among different sexes and races in larger well-controlled cohorts.

The authors acknowledge Anastasiya Matveyenko<sup>1</sup> and Srinivas Cherukuri<sup>1</sup> for their assistance with study conduction and laboratory measurements.

## AUTHOR CONTRIBUTIONS

Conception and design: MP, GS

Analysis and interpretation: MP, YL, GS

Data collection: MP, MH, RN, VP, NM, DB

Writing the article: MP, GS

Critical revision of the article: MP, MH, RN, VP, NM, DB, YL, GS

Final approval of the article: MP, MH, RN, VP, NM, DB, YL, GS

Statistical analysis: MP, YL

Obtained funding: GS

Overall responsibility: GS

## DISCLOSURES

None.

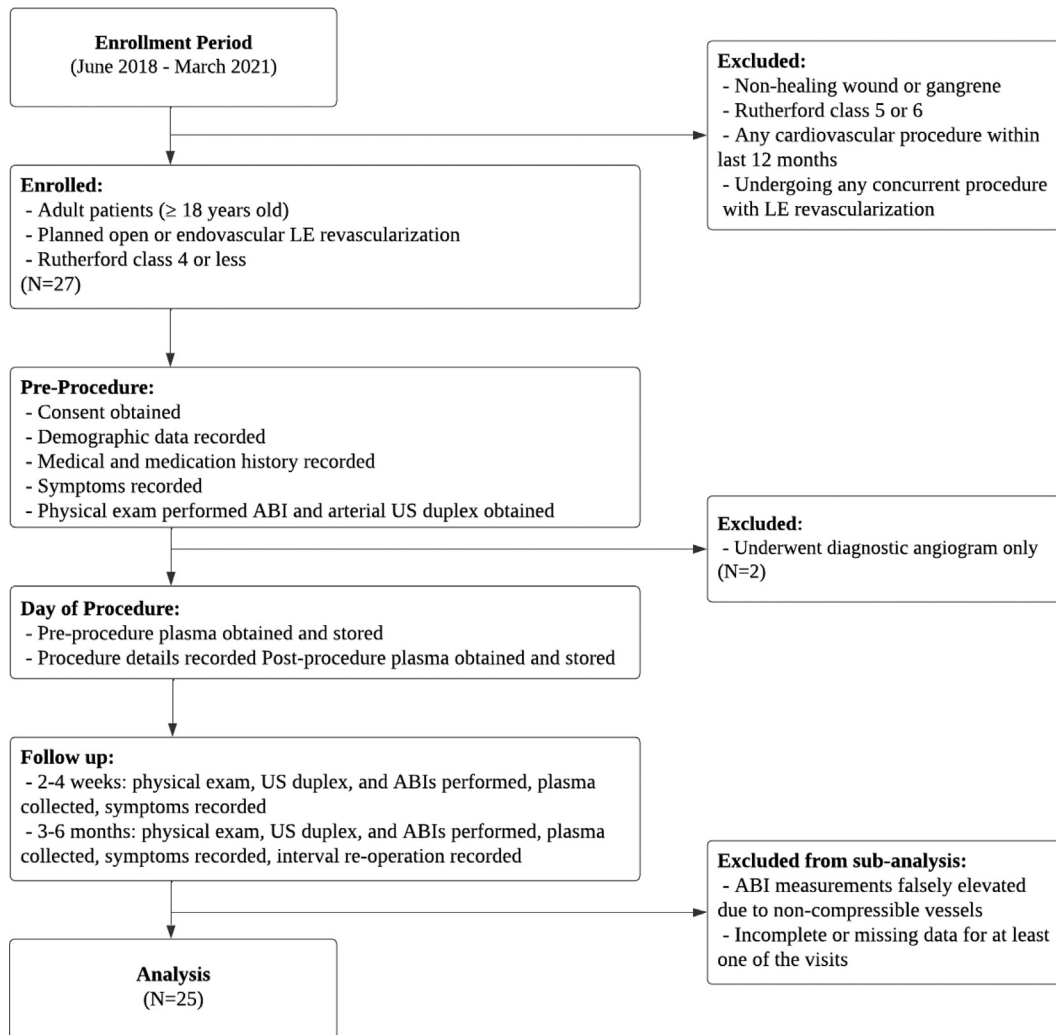
## REFERENCES

1. Reyes-Soffer G, Ginsberg HN, Berglund L, et al. Lipoprotein(a): a genetically determined, causal, and prevalent risk factor for atherosclerotic cardiovascular disease: a scientific statement from the American heart association. *Arterioscler Thromb Vasc Biol.* 2022;42:e48–e60.
2. Tomoi Y, Takahara M, Soga Y, et al. Impact of high lipoprotein(a) levels on clinical outcomes following peripheral endovascular therapy. *JACC Cardiovasc Interv.* 2022;15:1466–1476.
3. Verwer MC, Waissi F, Mekke JM, et al. High lipoprotein(a) is associated with major adverse limb events after femoral artery endarterectomy. *Atherosclerosis.* 2022;349:196–203.
4. Tsimikas S. A test in context: lipoprotein(a): diagnosis, prognosis, controversies, and emerging therapies. *J Am Coll Cardiol.* 2017;69:692–711.
5. Tsimikas S, Kiechl S, Willeit J, et al. Oxidized phospholipids predict the presence and progression of carotid and femoral atherosclerosis and symptomatic cardiovascular disease: five-year prospective results from the Bruneck study. *J Am Coll Cardiol.* 2006;47:2219–2228.
6. Reyes-Soffer G, Westerterp M. Beyond Lipoprotein(a) plasma measurements: lipoprotein(a) and inflammation. *Pharmacol Res.* 2021;169:105689.
7. Schmidt K, Noureen A, Kronenberg F, Utermann G. Structure, function, and genetics of lipoprotein (a). *J Lipid Res.* 2016;57:1339–1359.
8. Masson W, Lobo M, Barbagelata L, Molinero G, Bluro I, Nogueira JP. Elevated lipoprotein (a) levels and risk of peripheral artery disease outcomes: a systematic review. *Vasc Med.* 2022;27:385–391.
9. Dieplinger B, Lingenhel A, Baumgartner N, et al. Increased serum lipoprotein(a) concentrations and low molecular weight phenotypes of apolipoprotein(a) are associated with symptomatic peripheral arterial disease. *Clin Chem.* 2007;53:1298–1305.
10. Molgaard J, Klausen IC, Lassvik C, Faergeman O, Gerdes LU, Olsson AG. Significant association between low-molecular-weight apolipoprotein(a) isoforms and intermittent claudication. *Arterioscler Thromb.* 1992;12:895–901.
11. Ezhov MV, Safarova MS, Afanasieva OI, Kukharchuk VV, Pokrovsky SN. Lipoprotein(a) level and apolipoprotein(a) phenotype as predictors of long-term cardiovascular outcomes after coronary artery bypass grafting. *Atherosclerosis.* 2014;235:477–482.
12. Kandelouei T, Abbasifard M, Imani D, et al. Effect of statins on serum level of hs-CRP and CRP in patients with cardiovascular diseases: a systematic review and meta-analysis of randomized controlled trials. *Mediators Inflamm.* 2022;2022:8732360.
13. Sepeshri Z, Masoumi M, Ebrahimi N, et al. Atorvastatin, losartan and captopril lead to upregulation of TGF-beta, and downregulation of IL-6 in coronary artery disease and hypertension. *PLoS One.* 2016;11:e0168312.
14. Biscetti F, Ferraro PM, Hiatt WR, et al. Inflammatory cytokines associated with failure of lower-extremity endovascular revascularization (LER): a prospective study of a population with diabetes. *Diabetes Care.* 2019;42:1939–1945.
15. Tsimikas S, Karwatowska-Prokopczuk E, Gouni-Berthold I, et al. Lipoprotein(a) reduction in Persons with cardiovascular disease. *N Engl J Med.* 2020;382:244–255.

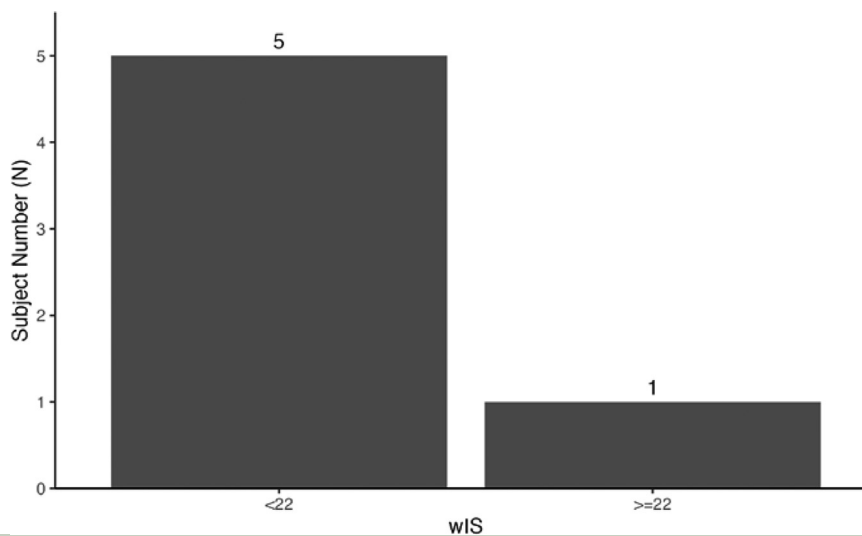
16. Mohammedi K, Pigeure M, Bosch J, Yusuf S, Gerstein HC. Arm and ankle blood pressure indices, and peripheral artery disease, and mortality: a cohort study. *Eur Heart J*. 2024;45:1738–1749.
17. Ioannis KECKP, Nicola Sartori Mean and median bias reduction in generalized linear models. *Stat Comput*. 2020;30:43–59.
18. European Society of Hypertension-European Society of Cardiology Guidelines C. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens*. 2003;21:1011–1053.
19. Yap T, Silickas J, Weerakkody R, et al. Predictors of outcome in diabetic patients undergoing infrapopliteal endovascular revascularization for chronic limb-threatening ischemia. *J Vasc Surg*. 2022;75:618–624.
20. Giovanetti F, Gargiulo M, Laghi L, et al. Lipoprotein(a) and other serum lipid subfractions influencing primary patency after infrainguinal percutaneous transluminal angioplasty. *J Endovasc Ther*. 2009;16:389–396.
21. Yanaka K, Akahori H, Imanaka T, et al. Impact of lipoprotein(a) levels on primary patency after endovascular therapy for femoropopliteal lesions. *Heart Vess*. 2023;38:171–176.
22. Fudim M, Hopley CW, Huang Z, et al. Association of hypertension and arterial blood pressure on limb and cardiovascular outcomes in symptomatic peripheral artery disease: the EUCLID trial. *Circ Cardiovasc Qual Outcomes*. 2020;13:e006512.
23. Arinze N, Farber A, Levin SR, et al. Perioperative outcomes after lower extremity bypass and peripheral vascular interventions in patients with morbid obesity and superobesity. *J Vasc Surg*. 2020;71:567–574.e564.
24. Dieter RS, Tomasson J, Gudjonsson T, et al. Lower extremity peripheral arterial disease in hospitalized patients with coronary artery disease. *Vasc Med*. 2003;8:233–236.
25. Hishikari K, Hikita H, Nakamura S, et al. Usefulness of lipoprotein(a) for predicting clinical outcomes after endovascular therapy for aortoiliac atherosclerotic lesions. *J Endovasc Ther*. 2017;24:793–799.
26. Guedon AF, De Fremerville JB, Mirault T, et al. Association of lipoprotein(a) levels with incidence of major adverse limb events. *JAMA Netw Open*. 2022;5:e2245720.
27. Pearson GJ, Thanassoulis G, Anderson TJ, et al. 2021 Canadian cardiovascular society guidelines for the management of dyslipidemia for the Prevention of cardiovascular disease in adults. *Can J Cardiol*. 2021;37:1129–1150.
28. Kronenberg F, Mora S, Stroes ESG, et al. Lipoprotein(a) in atherosclerotic cardiovascular disease and aortic stenosis: a European Atherosclerosis Society consensus statement. *Eur Heart J*. 2022;43:3925–3946.
29. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: executive summary: a report of the American college of cardiology/American heart association task force on clinical practice guidelines. *J Am Coll Cardiol*. 2019;73:3168–3209.
30. Viney NJ, Yeang C, Yang X, Xia S, Witztum JL, Tsimikas S. Relationship between "LDL-C", estimated true LDL-C, apolipoprotein B-100, and PCSK9 levels following lipoprotein(a) lowering with an antisense oligonucleotide. *J Clin Lipidol*. 2018;12:702–710.
31. Sniderman AD, Thanassoulis G, Glavinovic T, et al. Apolipoprotein B particles and cardiovascular disease: a narrative review. *JAMA Cardiol*. 2019;4:1287–1295.
32. Criqui MH, Matsushita K, Aboyans V, et al. Lower extremity peripheral artery disease: contemporary epidemiology, management gaps, and future directions: a scientific statement from the American heart association. *Circulation*. 2021;144:e171–e191.
33. Matveyenko A, Matienzo N, Ginsberg H, et al. Relationship of apolipoprotein(a) isoform size with clearance and production of lipoprotein(a) in a diverse cohort. *J Lipid Res*. 2023;64:100336.
34. Kraft HG, Lingenhel A, Kochl S, et al. Apolipoprotein(a) kringle IV repeat number predicts risk for coronary heart disease. *Arterioscler Thromb Vasc Biol*. 1996;16:713–719.
35. Boffa MB, Koschinsky ML. Lipoprotein (a): truly a direct prothrombotic factor in cardiovascular disease? *J Lipid Res*. 2016;57:745–757.
36. Caplice NM, Panetta C, Peterson TE, et al. Lipoprotein (a) binds and inactivates tissue factor pathway inhibitor: a novel link between lipoproteins and thrombosis. *Blood*. 2001;98:2980–2987.
37. Patel AP, Wang M, Pirruccello JP, et al. Lp(a) (Lipoprotein[a]) concentrations and incident atherosclerotic cardiovascular disease: new insights from a large national biobank. *Arterioscler Thromb Vasc Biol*. 2021;41:465–474.
38. Sepehri Z, Masoumi M, Ebrahimi N, et al. Correction: atorvastatin, losartan and captopril lead to upregulation of TGF-beta, and downregulation of IL-6 in coronary artery disease and hypertension. *PLoS One*. 2017;12:e0172404.
39. Bertoia ML, Pai JK, Lee JH, et al. Oxidation-specific biomarkers and risk of peripheral artery disease. *J Am Coll Cardiol*. 2013;61:2169–2179.
40. Patel SM, Bonaca MP, Morrow DA, et al. Lipoprotein(a) and benefit of antiplatelet therapy: insights from the PEGASUS-TIMI 54 trial. *JACC Adv*. 2023;2:100675.
41. Cui K, Yin D, Zhu C, et al. How do lipoprotein(a) concentrations affect clinical outcomes for patients with stable coronary artery disease who underwent different dual antiplatelet therapy after percutaneous coronary intervention? *J Am Heart Assoc*. 2022;11:e023578.
42. Marlys L, Koschinsky AB, Boffa MB, et al. A focused update to the 2019 NLA scientific statement on use of lipoprotein(a) in clinical practice. *J Clin Lipidol*. 2024;18:e308–e319.

Submitted Feb 2, 2024; accepted Jun 2, 2024.





**Supplementary Fig 1 (online only).** Anthropometric and Biomarker Assay, and Lower Extremity Arterial Revascularization (ABALLEAR) study Consolidated Standards of Reporting Trials (CONSORT) flow diagram. *ABI*, Ankle-brachial index; *LE*, lower extremity; *US*, ultrasound.



**Supplementary Fig 2 (online only).** Apolipoprotein (a) (apo(a)) weighted isoform sizes (wIS) in reoperative patients. Subject number (n) underwent reoperation at 3 to 6 months.

**Supplementary Table (online only).** Individual subject data

Subject number	Lp(a), nmol/L	Isoform 1	% Isoform 1	Isoform 2	%Isoform 2	wIS	SRRE
1	300.99	14	8	25	18	15.98	B
2	97.10	17	97	21	3	17.12	W
3	92.56	20	78	31	22	22.42	W
4	3.40	19	82	30	18	20.98	B
5	4.12	27	100			27	B
6	73.82	14	78	26	22	16.64	B
7	193.26	18	100			18	W
8	190.63	13	100			13	W
9	228.60	18	82	24	18	19.08	H
10	43.91	28	96	34	4	28.24	B
11	397.62	17	100			17	H
12 <sup>a</sup>	305.28	16	100			16	W
13	108.34	13	91	18	9	13.45	W
14	448.08	17	100			17	W
15	332.96	14	90	13	10	13.9	H
16	42.62	13	99	29	1	13.16	W
17	313.38	17	100			17	B
18	<2.80	15	100			15	W
19	63.84	24	100			24	B
20	42.47	19	84	24	16	19	H
21	156.73	19	100			19	W
22	67.04	23	100			23	H
23	7.05	15	81	32	19	18.23	W
24	143.43	21	100			21	W
25	556.02	14	99	29	1	14.15	B

B, Black; H, Hispanic; Lp(a), lipoprotein(a); SRRE, self-reported race/ethnicity; W, White; wIS, weighted isoform size. Large isoform  $\geq 22$ ; small isoform  $< 22$ .

<sup>a</sup>Calculated low density lipoprotein cholesterol not reported (triglyceride levels  $>400$  mg/dL).