

High Lipoprotein(a) Levels and Small Apolipoprotein(a) Size Prospectively Predict Cardiovascular Events in Dialysis Patients

J. Craig Longenecker,* Michael J. Klag,* Santica M. Marcovina,[†] Yong-Mei Liu,* Bernard G. Jaar,* Neil R. Powe,* Nancy E. Fink,* Andrew S. Levey,[‡] and Josef Coresh*

*Johns Hopkins University, Baltimore, Maryland; [†]Northwest Lipid Research Laboratories, University of Washington, Seattle, Washington; and [‡]Tufts-New England Medical Center, Boston, Massachusetts

Lipoprotein(a) [Lp(a)] levels are increased in dialysis patients, suggesting that they may play a role in the elevated atherosclerotic cardiovascular disease (ASCVD) risk in this population. Few prospective studies of Lp(a) level, apolipoprotein(a) [apo(a)] size, and ASCVD have been performed in the dialysis population. An inception cohort of 833 incident dialysis patients were followed prospectively. Baseline Lp(a) was measured by apo(a) size-independent ELISA and apo(a) size by Western blot after SDS-agarose gel electrophoresis. A combined prospective nonfatal and fatal ASCVD end point included myocardial infarction, coronary revascularization, cerebrovascular accident, carotid endarterectomy, peripheral revascularization, gangrene, or limb amputation. Survival analyses were performed with adjustment for baseline demographics, comorbid conditions, ASCVD risk factors, albumin, lipids, and C-reactive protein. Median follow-up was 27.4 mo, with 297 ASCVD events, 130 non-ASCVD deaths, and seven losses to follow-up over 1649 person-years. In multivariate Cox regression models, both high Lp(a) concentration (≥ 53 nmol/L) and low molecular weight (LMW) apo(a) isoforms (≤ 22 Kringle-IV repeats) predicted ASCVD events (relative hazard [RH] = 1.38, $P = 0.02$; RH = 1.58, $P < 0.0005$, respectively). In models that included both Lp(a) concentration and apo(a) size, only apo(a) size remained associated with ASCVD. Among those with both LMW apo(a) and Lp(a) level > 123 nmol/L, the relative hazard (RH) of ASCVD was 1.73 ($P < 0.0005$), compared with high molecular weight apo(a) and Lp(a) level < 123 nmol/L. No interactions by age, race, gender, diabetes, or ASCVD were present. Both LMW apo(a) size and high Lp(a) level predict ASCVD risk in dialysis patients, but the association of ASCVD with LMW isoforms is stronger than the association with high Lp(a) concentration.

J Am Soc Nephrol 16: ???-???, 2005. doi: 10.1681/ASN.2004110922

Patients with ESRD have a greatly elevated risk for atherosclerotic cardiovascular disease (ASCVD). This increased risk is only partially explained by traditional risk factors associated with ESRD (1–4), prompting interest in novel ASCVD risk factors, such as lipoprotein(a) [Lp(a)], levels of which are elevated in ESRD (5). Lp(a) is composed of an LDL particle covalently bonded to apolipoprotein(a) [apo(a)], a glycoprotein with a highly variable number of Kringle-IV (K-IV) units related to a polymorphism encoded in the apo(a) gene. Lp(a) levels are inversely related to apo(a) isoform size (6).

Many previous studies in the general population have found that high Lp(a) levels (7) and small apo(a) size (8–12) are associated with ASCVD. The few studies of Lp(a) in ESRD have provided conflicting results (13–15), with the only prospective study to evaluate both Lp(a) and apo(a) size reporting that

small apo(a) size but not Lp(a) level is associated with increased ASCVD (13).

The Choices for Healthy Outcomes in Caring for ESRD (CHOICE) Study, a prospective study of outcomes among black and white incident dialysis patients, previously found that small apo(a) size but not Lp(a) level was associated with total mortality (16). The current study, based on the CHOICE cohort, tested the *a priori* hypothesis that small apo(a) size but not high Lp(a) level is associated with prospectively ascertained ASCVD in a national, biracial cohort of patients who begin dialysis.

Materials and Methods

Study Design and Population

The CHOICE Study enrolled 1041 participants in 19 states from 81 dialysis clinics associated with Dialysis Clinic, Incorporated (DCI; Nashville, TN; $n = 923$ from 79 clinics), New Haven CAPD (New Haven, CT; $n = 86$ from one clinic), or Saint Raphael's Hospital (New Haven, CT; $n = 32$ from one clinic) from October 1995 to June 1998. Enrollment occurred a median of 1.6 mo after first dialysis (98% within 4 mo). Blood was obtained only at the DCI clinics, and samples were available for determination of Lp(a) level and apo(a) size for 872 (93.6%) of the 923 DCI participants; 833 (90.2%) were eligible for Lp(a)-related analysis. Enrollment criteria included initiation of dialysis in the preceding 3 mo, ability to give written informed consent, age over 17 yr, and ability to speak English or Spanish. The Johns Hopkins University School of Medicine Institutional Review Board approved the protocol.

Received November 9, 2004. Accepted March 2, 2005.

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. J. Craig Longenecker, Department of Community Medicine, Kuwait University Faculty of Medicine, P.O. Box 24923, Safat, 13110; Kuwait. Phone: 011-965-531-2000x 6534; Fax: 011-965-533-8948; E-mail: [jlongene@hsc.edu.kw](mailto: jlongene@hsc.edu.kw)

The funding sources had no role in the collection, analysis, or interpretation of the data and had no role in writing the report or in the decision to submit this article.

J.C.L. is currently affiliated with Kuwait University, Safat, Kuwait.

Data Collection

Baseline comorbidity was ascertained using a validated comorbidity score (range 0 to 3) derived from the Index of Co-Existent Disease (ICED) as described previously (17). Baseline ASCVD was defined as any history of myocardial infarction, cardiac revascularization procedure, stroke, carotid endarterectomy, extremity gangrene or peripheral revascularization procedure, limb amputation, or abdominal aortic aneurysm repair.

A composite ASCVD outcome was composed of the first cardiovascular event (fatal or nonfatal) during follow-up, including the same events as ascertained for the baseline ASCVD. Hospital records were requested for all deaths and for potential nonfatal events when any of four sources (quarterly dialysis nurse assessment, annual dialysis clinic record review, annual patient questionnaire, or periodic Health Care Financing Administration [HCFA] billing data) indicated a hospitalization for a potential ASCVD event or for congestive heart failure. A study physician reviewed all charts, flagging charts with a possible event for review by the ASCVD Outcomes Committee. Two ASCVD Outcome Committee physicians then independently reviewed all records with a potentially positive ASCVD event. Any disagreement between the two independent reviewers was adjudicated by a third reviewer.

All nonfatal events had specific written criteria for classification (myocardial infarction: history, EKG, and enzymes; stroke: symptoms, physical examination, and radiologic criteria; surgical procedures: operative note or date documented in the medical record). Depending on the combination of data observed, events were coded as "definite," "probable," "suspect," or "no event." Hospitalization charts were re-

viewed for 177 (92%) of 192 nonfatal events. The 15 events without an available hospital record were coded on the basis of the agreement of two of the four sources listed above, with a condition that one of the sources included the HCFA discharge diagnosis. The κ statistic between the two initial reviewers regarding the strength of diagnosis (definite, probable, suspect, and none) was 0.86 for myocardial infarction and 0.85 for cerebrovascular accident. After it became apparent that the agreement on strength of diagnosis between two reviewers was essentially 100% for procedure events, a single review was deemed sufficient for accurate coding. "Definite" and "probable" events were included as events in the final analysis.

Vital status is verified actively every 3 mo, and records are sent to the coordinating center upon a participant's death. Fatal outcome events were adjudicated by two independent physicians using a written algorithm for ASCVD cause of death ascertainment (see Table 1 for ASCVD cause of death classification codes modified from the HEMO Study [18]). The same criteria used for nonfatal events were used for fatal events. Medical records were available and reviewed for 92 (62%) of the 148 in-hospital deaths. Out-of-hospital deaths and in-hospital deaths with no chart available were coded according to the HCFA Death Notification Form (see Table 2). Agreement between the two independent reviewers on ASCVD causes of death was excellent ($\kappa = 0.88$ for coronary artery disease, 0.92 for cerebrovascular disease, and 0.82 for peripheral vascular disease).

Serum was collected and stored at -80°C . Sample draw occurred at a median of 4.4 mo after first dialysis (95% within 6.8 mo). Lp(a) concentration was measured by a direct binding double mAb-based ELISA, as previously reported (6). The detection antibody is directed to

Table 1. Atherosclerotic cardiovascular disease causes of death classification codes (modified from the HEMO Study)^a

Code	Description
CHD codes	
01DA	Sudden death as a result of CHD (sudden death in setting of previous CHD event)
01DB	MI
01DE	Other forms of ischemic heart disease
01DE-1	History positive for MI within 4 wk of death
01DE-2	Chest pain was present within 72 h of death
01DE-3	Congestive heart failure present at time of death, with a history of CHD
01DE-4	Lethal arrhythmia (not sudden) with history of coronary artery disease, but not qualifying for 01DE-1 to 01DE-3
01DH	Death from complication of cardiac revascularization
Cerebrovascular disease codes	
06DA	Cerebrovascular accident, thrombotic
06DF	Death from complication of carotid endarterectomy procedure
PVD codes	
07DB	Peripheral vascular disease (coded if any of the following are positive)
07DA	Hemorrhage from ruptured vascular aneurysm
07DE	Abdominal aortic aneurysm
07DF	Thoracic aortic aneurysm
07DG	Aortic aneurysm (not specified as abdominal aortic aneurysm or thoracic aortic aneurysm)
07DJ	Mesenteric ischemia or infarction/ischemic bowel
07DK	Gangrene with or without septicemia-shock as a result of PVD
07DN	Death from complication of a PVD procedure

^aAll codes are assigned using specific designated criteria. CHD, coronary heart disease; MI, myocardial infarction; PVD, peripheral vascular disease.

Table 2. Atherosclerotic cardiovascular disease causes of death on HCFA Death Notification Form (used for out-of-hospital deaths and in-hospital deaths for which no hospital record was available)^a

Code	Description
CHD death codes	
23	MI, acute
26	Atherosclerotic heart disease
28	Cardiac arrhythmia (coded as an ASCVD death if the patient had a previous CHD event)
29	Cardiac arrest, cause unknown (coded as an ASCVD death if the patient had a previous CHD event)
Cerebrovascular disease death codes	
36	Cerebrovascular accident
37	Ischemic brain damage
PVD death codes	
41	Hemorrhage from ruptured vascular aneurysm
44	Mesenteric infarction/ischemic bowel
51	Septicemia, as a result of PVD, gangrene

^aASCVD, atherosclerotic cardiovascular disease; HCFA, Health Care Financing Administration.

a nonrepeating epitope present in apo(a) K-IV type 9, making this assay insensitive to apo(a) size. Lp(a) concentrations were expressed in nmol/L. The analytical coefficient of variation of Lp(a), based on five duplicate samples of varying Lp(a) concentrations (12 to 120 nmol/L) in each ELISA plate, ranged from 4.0 to 6.7%.

Apo(a) isoforms were characterized using a high-resolution SDS-agarose gel electrophoresis method followed by immunoblotting, as previously reported (19). We used a size designation related to each isoform's number of K-IV repeats (19,20). The coefficient of variation for apo(a) size in the CHOICE cohort was 11.7% ($n = 49$). An exact match of the smallest allele size for the 49 blindly split samples was present for 48.9%, and a match ± 1 repeat was present for 93.8%.

Statistical Analyses

Statistical analyses were performed with Stata (version 6.0). The Mann-Whitney *U* test was used to test for differences in median values of skewed variables. Lp(a) was dichotomized at the median (52.5 nmol/L) and log-transformed when analyzed continuously. Low molecular weight (LMW) apo(a) isoforms were designated by convention as ≤ 22 K-IV repeats. Both were also analyzed by quartile and tested for trend across quartiles. In addition, some analyses evaluated an Lp(a) cutoff of ≤ 206 nmol/L (≤ 90 th percentile) and an apo(a) size cutoff of ≤ 16 K-IV repeats (≤ 10 th percentile). All analyses of apo(a) size used the predominantly expressed isoform.

Survival time was defined as time from blood draw to outcome event or censoring. The time scale used in the analysis was time from first dialysis, with staggered entry at time of exposure ascertainment. Of the 872 individuals with Lp(a) data, 29 were excluded from analysis owing to a new ASCVD event between enrollment and Lp(a) determination, and 10 individuals were excluded to allow for left truncation at 4.0 mo for the purpose of producing more stable Kaplan-Meier curves.

Several groups of covariates were selected *a priori* for inclusion in Cox regression models. Group 1 (demographics and modality) included age, race, gender, and baseline dialysis modality. Group 2 (comorbid conditions) added the ICED score, cause of renal disease, and baseline ASCVD. Group 3 (other CVD risk factors) added total cholesterol, HDL cholesterol, smoking status, and systolic BP. Group 4 (nutrition) added albumin, body mass index, and creatinine. C-reactive

protein (CRP) was also added to group 4 in separate models. *A priori* stratified analyses investigating interactions were also performed by age, race, gender, dialysis modality, diabetes, prevalent ASCVD, LDL cholesterol, and CRP level. The proportionality assumption of the Cox models was tested using $-\ln[-\ln(\text{survival})]$ curves and regression of scaled Schoenfeld residuals on functions of time. No variables were found to violate model assumptions.

Those with prevalent ASCVD outcomes were included in the primary analysis for several reasons. First, an analysis that was restricted to those without prevalent ASCVD found a similar association to that of the analysis that was restricted to those with a history of ASCVD (there was no interaction). Importantly, those with previous ASCVD events remain at risk (and higher risk) for future ASCVD events. There is no scientific evidence that Lp(a) ceases to be a risk factor for future events after a first event. Furthermore, because the prevalence of ASCVD is almost 50%, to exclude prevalent cases significantly reduces the statistical power of the analysis. Excluding so many in the cohort would also likely render the results less generalizable to the whole dialysis population. Last, in the dialysis population, those without a history of CVD events have a far higher level of atherosclerosis than is found among those in the general population without a history of CVD. Therefore, the difference in the degree of atherosclerosis between those with and without CVD in the dialysis population is not as great as would be seen in other populations. For all of these reasons, it was decided to include those with prevalent CVD and to adjust for this factor in the primary analyses.

Results

Median follow-up was 27.4 mo, with 297 ASCVD events (192 nonfatal and 105 fatal) during 1649 person-years at risk. A total of 130 (15.6%) died from non-ASCVD causes, seven (0.8%) were lost to follow-up, 144 (17.3%) were censored upon renal transplantation, and the remainder were followed until the end of follow-up.

Table 3 shows that age, gender, and race in the CHOICE cohort are similar to the contemporary US dialysis population (1997 US Renal Data System data) (21). Table 4 demonstrates

Table 3. Characteristics of the US dialysis population (USRDS data) and baseline characteristics of the CHOICE cohort^a

Characteristic	1997 USRDS Incident Dialysis Patients (<i>n</i> = 79,102)	CHOICE Participants (<i>n</i> = 833)
Median age (years [IQR])	61	59.2 (46.3, 68.9)
Female gender (<i>n</i> [%])	47	391 (46.9)
Race (<i>n</i> [%])		
white	65	534 (64.1)
black	29	255 (30.6)
other	6	44 (5.3)
Dialysis modality (<i>n</i> [%])		
HD	87	675 (81.0)
PD	13	158 (19.0)
ESRD cause (<i>n</i> [%])		
diabetes	42	392 (47.1)
HTN	25	146 (17.5)
GN	9	130 (15.6)
other	23	44 (19.8)
Baseline CVD (<i>n</i> [%])		352 (42.3)
Diabetes (<i>n</i> [%])		453 (54.4)
Median Lp(a) (nmol/L [IQR])		
all		52.5 (16.2 to 121.9)
whites		36.3 (10.6 to 106.0)
blacks		86.9 (46.9 to 148.1)
Median apo(a) size (K-IV repeats [IQR])		
all		24 (19 to 28)
whites		25 (19 to 29)
blacks		23 (20 to 27)
Median time from first dialysis to enrollment (mo [IQR])		1.6 (0.9 to 2.5)
Median time from first dialysis to Lp(a) determination (mo [IQR])		4.4 (3.7 to 5.1)
Median follow-up (mo [range])		33.8 (0 to 63.9)

^aUSRDS, US Renal Data System; CHOICE, Choices for Healthy Outcomes in Caring for ESRD; IQR, interquartile range; HD, hemodialysis; PD, peritoneal dialysis; HTN, hypertension; GN, glomerulonephritis; CVD, cardiovascular disease; K-IV, Kringle-IV.

that those who were censored at renal transplantation (*n* = 163), compared with those who remained under follow-up, were significantly younger and included more men, whites, participants on peritoneal dialysis, participants with a low ICED comorbidity score, and causes of renal disease other than diabetes and hypertension. The transplant group had a lower Lp(a) level (45.0 *versus* 54.4 nmol/L) but did not differ significantly by apo(a) subtype.

Figure 1A presents unadjusted Kaplan-Meier curves showing a marginal association between Lp(a) level and ASCVD events among whites but no association among blacks. A stronger association exists between LMW apo(a) isoform size and ASCVD in both whites and blacks (Figure 1B).

In multivariate Cox models, Lp(a) level >52.5 nmol/L was independently associated with a 30 to 40% increased risk for ASCVD events (Table 5). A 60 to 90% increased risk for ASCVD was seen for Lp(a) levels ≥206 nmol/L (90th percentile), compared with Lp(a) <206 nmol/L. LMW apo(a) size was associated with a 60 to 90% increase risk for ASCVD events, with

adjustment for the same covariates, and apo(a) size ≤16 K-IV repeats (≤10th percentile) was associated with a 40 to 100% increase in ASCVD risk, compared with apo(a) size >16 K-IV repeats (Table 6).

Figure 2 presents ASCVD risk by Lp(a) concentration and apo(a) size quartile, showing that the adjusted risk for ASCVD events is significantly increased both with higher Lp(a) concentrations (A) and with smaller apo(a) size (B), although the association with smaller apo(a) size is stronger than the association with increased Lp(a) concentration. For Lp(a) ≥206 nmol/L (≥90th percentile) compared with the first quartile, the relative hazard (RH) was 1.71 (1.11–2.65; *P* = 0.015). For apo(a) isoforms ≤16 K-IV repeats (≤10th percentile) compared with the fourth apo(a) size quartile, the RH was 2.0 (1.27–3.14; *P* = 0.003).

The same associations were present when only individuals with no prevalent ASCVD were included in the analysis (*n* = 410 with 92 events; for LMW isoforms, RH = 1.82, *P* = 0.007; for Lp(a) ≥52.5 nmol/L, RH = 1.33, *P* = 0.23; and for Lp(a) ≥206.0

Table 4. Cohort characteristics, by transplant status^a

Characteristic	Nontransplant Group ^b (n = 682)	Transplant Group ^c (n = 144)	P Value
Median age (yr)	61.8	45.4	<0.0005
Race (%)			0.03
white	62.0	73.6	
black	32.4	23.6	
other	5.6	2.8	
Female gender (%)	48.8	36.8	0.009
Dialysis modality (%)			<0.0005
HD	83.7	69.4	
PD	16.3	30.6	
Cause of renal disease (%)			<0.0005
diabetes	50.6	31.9	
HTN	18.3	13.9	
GN	13.1	26.4	
other	18.0	27.8	
ICED comorbidity score (%)			<0.0005
level 0-1	28.9	56.9	
level 2	39.8	25.7	
level 3	31.2	17.4	
CVD (%)	47.0	20.8	<0.0005
Diabetes (%)	56.4	37.5	<0.0005
Median Lp(a) level (nmol/L)	54.4	45.0	0.06
Median apo(a) size	24	25	0.36

^aICED, Index of Co-Existent Disease.

^bThose who remained under active or passive follow-up until administrative censoring or death.

^cThose who remained under follow-up until transplantation.

nmol/L, RH = 2.53; $P = 0.003$]. The association was similar in whites ($n = 460$ with 188 events; for Lp(a) ≥ 52.5 nmol/L, RH = 1.44, $P = 0.02$; and for LMW apo(a) size, RH = 1.63, $P = 0.001$) and blacks, although the estimates for blacks were not statistically significant ($n = 230$ with 59 events; for Lp(a) ≥ 52.5 nmol/L, RH = 1.43, $P = 0.26$; and for LMW apo(a) size, RH = 1.44, $P = 0.24$). No significant interactions by age, race, gender, dialysis modality, diabetes, prevalent ASCVD, LDL cholesterol, and CRP level were present for either Lp(a) level or apo(a) size.

When high Lp(a) concentration (quartile 4 cutoff) and LMW apo(a) size are entered simultaneously into a Cox regression model, the effect of small apo(a) size remains (RH = 1.52; $P = 0.004$), whereas the effect of high Lp(a) concentration becomes insignificant (RH = 1.15; $P = 0.238$). Figure 3 demonstrates that there is no interaction between Lp(a) concentration and apo(a) size in the risk for ASCVD events and that the effect size of LMW apo(a) size is much larger than that of high Lp(a) concentration, when considered together. Similar associations were seen when Lp(a) level and apo(a) size were dichotomized at the median or the quartile 4 cutoff.

Discussion

Previous studies have found either high Lp(a) concentration or small apo(a) size but not both to be risk factors for ASCVD in the dialysis population. This prospective study of 833 incident dialysis patients found a moderate, independent associa-

tion between ASCVD and high Lp(a) concentration and a stronger association with LMW apo(a) isoform size. Although no multiplicative interaction was seen between small apo(a) size and high Lp(a) level, the group with LMW isoforms and Lp(a) concentration >123 nmol/L had the highest risk (RH = 1.73; $P < 0.0005$).

A large number of prospective studies in the general population have found an association between high Lp(a) level and coronary heart disease (7), but few prospective studies of apo(a) isoform size and CHD in the general population have been published (8,12). The largest nested case-control study of Lp(a), apo(a), and incident CHD in the general population ($n = 134$ cases) found an association between Lp(a) and CHD but not between apo(a) size and CHD, although the crude analysis showed a trend toward smaller apo(a) size among cases (12). Conversely, the Bruneck Study found that LMW isoform size was prospectively associated with advanced atherosclerosis, especially in the presence of elevated Lp(a) concentrations, and that Lp(a) concentration was associated with early atherosclerosis, but this was restricted to those with elevated LDL cholesterol levels (8).

Kronenberg *et al.* reported the only previously published prospective study that investigated both Lp(a) level and apo(a) size as risk factors for CHD in the dialysis population (13). They found that LMW isoform size, not Lp(a) level, was associated with the development of CHD ($n = 66$ CHD events; adjusted

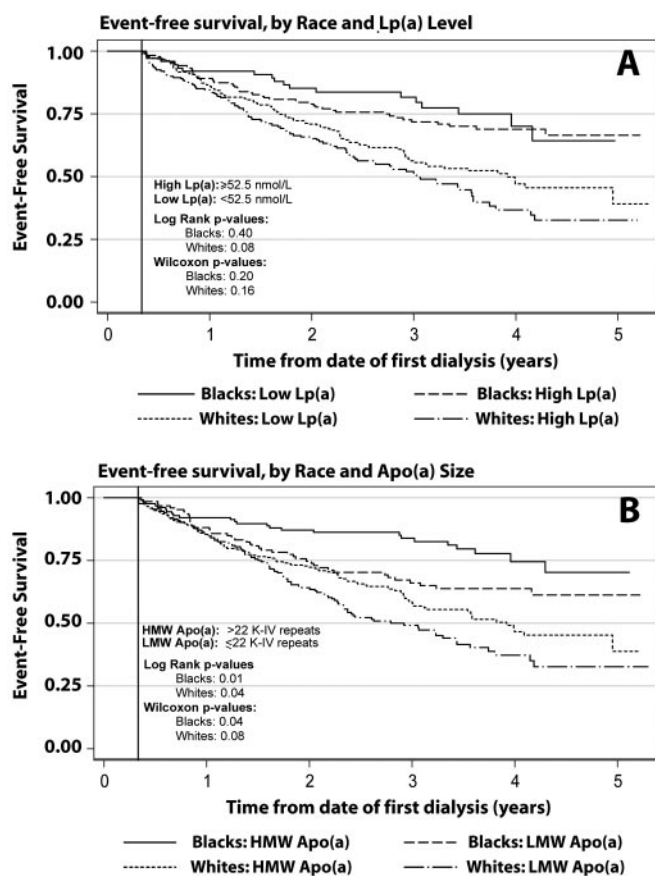


Figure 1. Kaplan-Meier survival curves showing time to combined fatal and nonfatal atherosclerotic cardiovascular disease (ASCVD) event, stratified by race, lipoprotein(a) [Lp(a)] level [low Lp(a) level < 52.5 nmol/L; high Lp(a) level ≥ 52.5 nmol/L; A], and apolipoprotein(a) [apo(a)] size [low molecular weight (LMW) apo(a) size ≤ 22 Kringle IV (K-IV) repeats; high molecular weight (HMW) apo(a) size > 22 K-IV repeats; B].

RH = 2.3; $P = 0.0008$). The same authors reported earlier that, among those with high molecular weight apo(a) isoforms, Lp(a) levels are much higher in hemodialysis patients than in apo(a) phenotype-matched population control subjects (17.2 versus 10.8 mg/dl; $P < 0.0001$), whereas among those with LMW isoforms, the Lp(a) level in ESRD patients is only slightly higher than that of phenotype-matched population control subjects with high Lp(a) levels (40.8 versus 36.9 mg/dl; $P = 0.14$) (5,22). If such a differential increase in Lp(a) by apo(a) subtype occurs as renal failure develops, then the association between Lp(a) and ASCVD would be altered toward the null, while maintaining the association between small apo(a) size and ASCVD. This hypothesis is supported by our study in that the association between Lp(a) and ASCVD was weaker than that seen with small apo(a) size. Our earlier finding that small apo(a) size but not Lp(a) level prospectively predicts total mortality also is consistent with the above hypothesized mechanism (16).

Other explanations are possible, however. Because apo(a) size remains the strongest predictor of Lp(a) levels in the ESRD

population, one would expect that Lp(a) would still predict ASCVD to some extent, which this study demonstrates. Several features may explain why our study found an association between Lp(a) and ASCVD whereas some other studies may not. First, most other studies recruited prevalent ESRD patients, leading to a potential survival bias. If a risk factor is associated with an outcome that can lead to death (e.g., death from myocardial infarction), then those with the highest levels tend to be underrepresented in a prevalent population, because they die sooner. This “survival” bias, which tends to bias associations toward the null, is particularly strong in the setting of extremely high mortality rates in the dialysis population. Because this study recruited all participants soon after initiation of dialysis, the Lp(a) association is less affected by such a bias, although it is probably still affected to some extent. Second, because the association between Lp(a) and ASCVD is moderate in magnitude, other studies with fewer outcome events may not have had the statistical power to find associations. Third, as shown in Table 4, there may be significant informative censoring associated with transplantation, which would affect a prevalent study population much more than a cohort of incident dialysis patients. Among the much healthier group that received a transplant, Lp(a) levels were lower than among the less healthy adherent group. This selective removal of healthier patients with lower Lp(a) levels biases the association of Lp(a) with ASCVD toward the null. Last, this study used an apo(a) size-independent assay for Lp(a), whereas other studies generally used assays that are sensitive to apo(a) size. Assays for which the antibody detects all K-IV repeats may result in misclassification of Lp(a) level. The larger the test sample apo(a) isoform size [i.e., in those with lower Lp(a) levels] relative to the apo(a) size of the reference standard, the more the Lp(a) mass value will be overestimated. The smaller the test sample apo(a) isoform size [i.e., in those with higher Lp(a) levels] relative to the apo(a) size of the reference standard, the more the Lp(a) mass value will be underestimated. This process results in a type of misclassification that biases relationships between Lp(a) and associated outcomes toward the null while leaving associations with apo(a) size intact. Apo(a) size-independent assays, such as used in this study, avoid this source of misclassification.

Some other studies have suggested that high Lp(a) levels are most atherogenic when present in conjunction with other ASCVD risk factors such as elevated LDL cholesterol (23) or elevated fibrinogen (24). However, in this study, no significant interactions by age, race, gender, diabetes, dialysis modality, prevalent ASCVD, LDL cholesterol, or CRP level were present for either Lp(a) level or apo(a) size.

The strengths of this study include the enrollment of incident dialysis patients; inclusion of a sample of blacks and whites from a wide geographic area; a high follow-up rate; the measurement of Lp(a) concentration, not mass; and the accuracy of the apo(a) assay. The most important limitation of this study is the problem of informative censoring as a result of the high transplantation rate (17.3%), which may have biased the Lp(a) results toward the null. This may partially explain why Lp(a) level is more weakly associated with ASCVD than is apo(a) size in this cohort. For apo(a) size, however, informative censoring

Table 5. Adjusted associations between Lp(a) concentration (nmol/L) and ASCVD events, by adjustment group^a

Adjustment Group	Model (N)	CVD Events (N)	Median Lp(a) Models Lp(a) ≥ 52.5 nmol/L versus Lp(a) < 52.5 nmol/L			90th Percentile Lp(a) Models Lp(a) ≥ 206 nmol/L versus Lp(a) < 206 nmol/L		
			HR	95% CI	P	HR	95% CI	P
Group 1 ^b	833	297	1.31	(1.03 to 1.65)	0.03	1.64	(1.17 to 2.31)	0.004
Group 2 ^c	832	296	1.25	(0.99 to 1.58)	0.07	1.51	(1.07 to 2.14)	0.02
Group 3 ^d	731	262	1.38	(1.06 to 1.79)	0.02	1.89	(1.30 to 2.75)	0.001
Group 4 ^e	675	242	1.44	(1.09 to 1.90)	0.01	1.89	(1.27 to 2.79)	0.002
Group 4+CRP	660	238	1.37	(1.03 to 1.82)	0.03	1.88	(1.25 to 2.81)	0.002

^aCI, confidence interval; CRP, C-reactive protein.

^bAdjusted for age, race (black, white, other), gender, and dialysis modality.

^cAdjusted for group 1 and baseline CVD, comorbidity score, and cause of renal disease.

^dAdjusted for group 2 and total cholesterol, HDL cholesterol quartile, smoking status, and systolic BP quartile.

^eAdjusted for group 3 and albumin, creatinine, and body mass index quartiles.

Table 6. Adjusted associations between apo(a) size (K-IV repeats) and CVD events, by adjustment group^a

Adjustment Group	Model (N)	CVD Events (N)	LMW Apo(a) Size Models Apo(a) ≤ 22 K-IV Repeats versus Apo(a) > 22 K-IV Repeats			10th Percentile Apo(a) Size Models Apo(a) ≤ 16 K-IV Repeats versus Apo(a) > 16 K-IV Repeats		
			HR	95% CI	P	HR	95% CI	P
Group 1 ^b	833	297	1.40	(1.11 to 1.75)	0.004	1.44	(1.01 to 2.06)	0.046
Group 2 ^c	832	296	1.42	(1.13 to 1.79)	0.003	1.39	(0.96 to 1.99)	0.08
Group 3 ^d	731	262	1.58	(1.23 to 2.03)	<0.0005	1.68	(1.13 to 2.49)	0.01
Group 4 ^e	675	242	1.57	(1.21 to 2.04)	0.001	1.90	(1.27 to 2.84)	0.002
Group 4+CRP	660	238	1.71	(1.31 to 2.25)	<0.0005	1.96	(1.30 to 2.96)	0.001

^aLMW, low molecular weight.

^bAdjusted for age, race (black, white, other), gender, and dialysis modality.

^cAdjusted for group 1 and baseline CVD, comorbidity score, and cause of renal disease.

^dAdjusted for group 2 and total cholesterol, HDL cholesterol quartile, smoking status, and systolic BP quartile.

^eAdjusted for group 3 and albumin, creatinine, and body mass index quartiles.

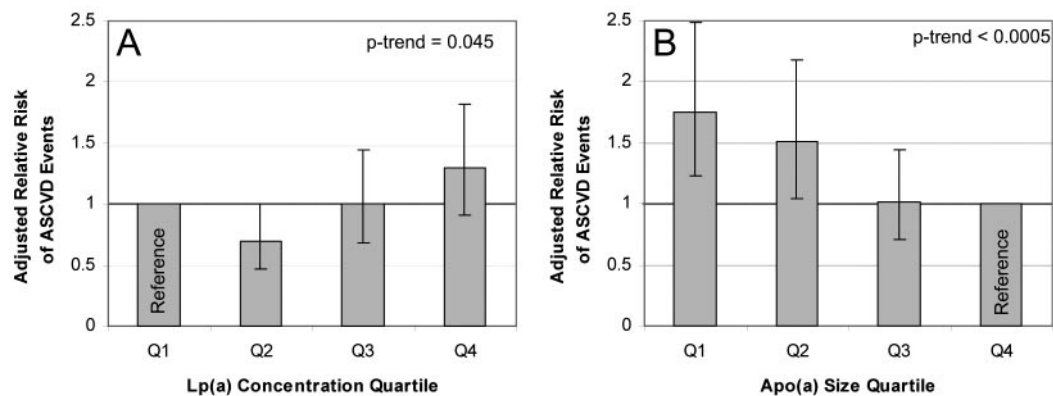


Figure 2. Adjusted relative risk (adjusted for group 3 covariates) of combined fatal and nonfatal ASCVD events, by Lp(a) quartile (A) and apo(a) size quartile (B).

is probably not a major issue because the distribution of apo(a) size did not differ by transplantation status.

Lp(a) may produce atherogenic effects through its action as a lipid or through inhibition of fibrinolysis by the apo(a) glyco-

protein (25). The apo(a) glycoprotein moiety displays an 80% homology with plasminogen (26) and inhibits fibrinolysis (27). Small apo(a) isoforms also have been found to bind more strongly to fibrin than larger isoforms (28), suggesting that

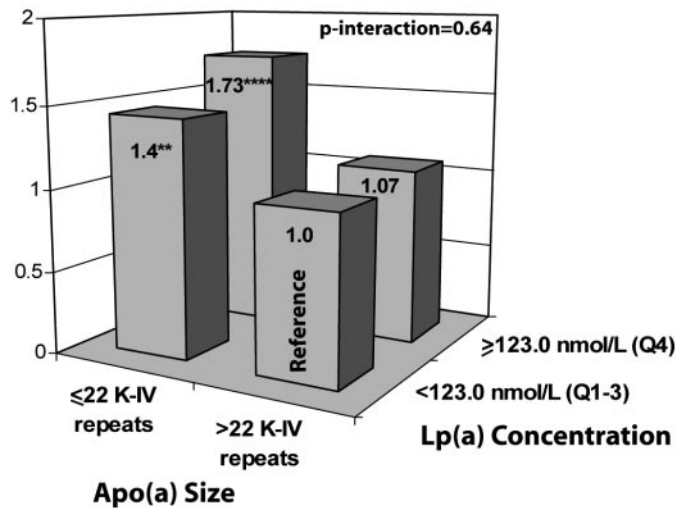


Figure 3. Adjusted ASCVD risk (adjusted for group 3 covariates) associated with apo(a) size and Lp(a) concentration among 833 dialysis patients. ** $P < 0.01$; **** $P < 0.0005$.

small apo(a) isoform size itself, not only the associated Lp(a) level, may be important in accelerating atherosclerosis. Nevertheless, in this mechanism, Lp(a) level would probably still be expected to play a significant role because more apo(a) glycoprotein particles would be available to inhibit fibrinolysis.

If the risk of ASCVD related to Lp(a) is mediated by both apo(a) isoform size and the attendant Lp(a) level, then Lp(a)-lowering therapy has potential to prevent ASCVD, particularly among those with LMW apo(a) isoforms. The finding that high Lp(a) levels are associated with an approximately 40% increase in ASCVD event rate suggests that successful lowering of Lp(a) level has potential to lower ASCVD risk in dialysis patients. The recent Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein(a) and Cardiovascular Disease (29) presented a brief review of treatment strategies to decrease Lp(a) levels, including niacin, ascorbic acid with L-lysine, estrogen, aspirin, statins, diet, and apheresis. Overall, Lp(a) level is difficult to lower, and most of the medications that do have an effect on Lp(a) levels also treat other atherogenic lipids. Sorting out the beneficial effect of Lp(a)-lowering medications beyond the cardiovascular benefit of such medications will require carefully designed, randomized, clinical trials.

Whether lowering Lp(a) levels is feasible or ultimately reduces ASCVD risk, elevated Lp(a) level or the presence of LMW apo(a) phenotype may be clinically useful in risk stratification and identification of dialysis patients who warrant more aggressive ASCVD prevention efforts. If part of the atherogenic effect of Lp(a) is mediated specifically through the antifibrinolytic action of LMW apo(a) isoforms, then future therapies that are designed specifically against the fibrin-binding action of LMW isoforms might also be effective. Further studies should explore whether the risk associated with LMW isoforms is mediated through the associated long-term elevation in Lp(a) levels or other mechanisms related to apo(a) size.

Acknowledgments

CHOICE was supported by R01-HS-08365 (Agency for Healthcare Research and Quality [AHRQ]) from June 1995 to May 2000 and is currently supported by R01-HL-62985 (National Heart, Lung, and Blood Institute [NHLBI]) and R01-DK-07024 (National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK]). Other grants supporting this research are R29-DK-48362 (NIDDK; J.C.), K24-DK-02856 (NIDDK; M.J.K.), K08-HL-03896 (NHLBI; J.C.L.), K24-DK-02643 (NIDDK; N.R.P.), American Heart Association Grant-in-Aid [J.C., principal investigator; Lp(a) and apo(a) assays], and National Center for Research Resources (National Institutes of Health) General Clinical Research Center grant M01-RR00052 (J.C., principal investigator; lipid assays). B.G.J. is the recipient of the Richard Ross Clinician Scientist Award from the Johns Hopkins School of Medicine.

We thank the CHOICE Study Cardiovascular Endpoint Committee. Current members are Bernard G. Jaar, MD, MPH; J. Craig Longenecker, MD, PhD; Josef Coresh, MD, PhD; Yongmei Liu, MD; Joseph A. Eustace, MD, MHS; Richard M. Ugarte, MD; and Melanie H. Katzman, MD, MHS. Former members of the Committee include Michael Klag, MD, MPH; Neil R. Powe, MD, MPH, MBA; Michael J. Choi, MD; Renuka Sothnathan, MD, MHS; and Caroline Fox, MD, MPH. Cardiovascular events adjudicators are Nancy E. Fink, MPH; and Laura C. Plantiga, ScM.

References

- Coresh J, Longenecker JC, Miller ER, Young HJ, Klag MJ: Epidemiology of cardiovascular risk factors in chronic renal disease. *J Am Soc Nephrol* 9[Suppl]: S24–S30, 1998
- Foley RN, Parfrey PS, Sarnak MJ: Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 32: S112–S119, 1998
- Special Report From the National Kidney Foundation Task Force on Cardiovascular Disease: Controlling the epidemic of cardiovascular disease in chronic renal disease: What do we know? What do we need to know? Where do we go from here? *Am J Kidney Dis* 32: S1–S199, 1998
- Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW: Kidney disease as a risk factor for development of cardiovascular disease: A statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 108: 2154–2169, 2003
- Kronenberg F, Konig P, Neyer U, Auinger M, Pribasniq A, Lang U, Reitinger J, Pinter G, Utermann G, Dieplinger H: Multicenter study of lipoprotein(a) and apolipoprotein(a) phenotypes in patients with end-stage renal disease treated by hemodialysis or continuous ambulatory peritoneal dialysis. *J Am Soc Nephrol* 6: 110–120, 1995
- Marcovina SM, Albers JJ, Gabel B, Koschinsky ML, Gaur VP: Effect of the number of apolipoprotein(a) kringle 4 domains on immunochemical measurements of lipoprotein(a). *Clin Chem* 41: 246–255, 1995
- Danesh J, Collins R, Peto R: Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. *Circulation* 102: 1082–1085, 2000
- Kronenberg F, Kronenberg MF, Kiechl S, Trenkwalder E, Santer P, Oberhollenzer F, Egger G, Utermann G, Willeit J: Role of lipoprotein(a) and apolipoprotein(a) phenotype in

- atherogenesis: Prospective results from the Bruneck study. *Circulation* 100: 1154–1160, 1999
9. Marcovina SM, Koschinsky ML: Lipoprotein(a) concentration and apolipoprotein(a) size: A synergistic role in advanced atherosclerosis? *Circulation* 100: 1151–1153, 1999
 10. Sandholzer C, Saha N, Kark JD, Rees A, Jaross W, Dieplinger H, Hoppichler F, Boerwinkle E, Utermann G: Apo(a) isoforms predict risk for coronary heart disease. A study in six populations. *Arterioscler Thromb* 12: 1214–1226, 1992
 11. Klausen IC, Sjol A, Hansen PS, Gerdes LU, Moller L, Lemming L, Schroll M, Faergeman O: Apolipoprotein(a) isoforms and coronary heart disease in men: A nested case-control study. *Atherosclerosis* 132: 77–84, 1997
 12. Wild SH, Fortmann SP, Marcovina SM: A prospective case-control study of lipoprotein(a) levels and apo(a) size and risk of coronary heart disease in Stanford Five-City Project participants. *Arterioscler Thromb Vasc Biol* 17: 239–245, 1997
 13. Kronenberg F, Neyer U, Lhotta K, Trenkwalder E, Auinger M, Pribasnig A, Meisl T, Konig P, Dieplinger H: The low molecular weight apo(a) phenotype is an independent predictor for coronary artery disease in hemodialysis patients: A prospective follow-up. *J Am Soc Nephrol* 10: 1027–1036, 1999
 14. Webb AT, Reaveley DA, O'Donnell M, O'Connor B, Seed M, Brown EA: Lipids and lipoprotein(a) as risk factors for vascular disease in patients on renal replacement therapy. *Nephrol Dial Transplant* 10: 354–357, 1995
 15. Cressman MD, Abood D, O'Neil J, Hoff HF: Lp(a) and premature mortality during chronic hemodialysis treatment. *Chem Phys Lipids* 67–68: 419–427, 1994
 16. Longenecker JC, Klag MJ, Marcovina SM, Powe NR, Fink NE, Giaculli F, Coresh J: Small apolipoprotein(a) size predicts mortality in end-stage renal disease: The CHOICE Study. *Circulation* 106: 2812–2818, 2003
 17. Miskulin DC, Meyer KB, Athienites NV, Martin AA, Terrin N, Marsh JV, Fink NE, Coresh J, Powe NR, Klag MJ, Levey AS: Comorbidity and other factors associated with modality selection in incident dialysis patients: The CHOICE Study. Choices for Healthy Outcomes in Caring for End-Stage Renal Disease. *Am J Kidney Dis* 39: 324–336, 2002
 18. Rocco MV, Yan G, Gassman J, Lewis JB, Ornt D, Weiss B, Levey AS: Comparison of causes of death using HEMO Study and HCFA end-stage renal disease death notification classification systems. The National Institutes of Health-funded Hemodialysis. Health Care Financing Administration. *Am J Kidney Dis* 39: 146–153, 2002
 19. Marcovina SM, Zhang ZH, Gaur VP, Albers JJ: Identification of 34 apolipoprotein(a) isoforms: Differential expression of apolipoprotein(a) alleles between American blacks and whites. *Biochem Biophys Res Commun* 191: 1192–1196, 1993
 20. Marcovina SM, Hobbs HH, Albers JJ: Relation between number of apolipoprotein(a) kringle 4 repeats and mobility of isoforms in agarose gel: Basis for a standardized isoform nomenclature. *Clin Chem* 42: 436–439, 1996
 21. United States Renal Data System: *USRDS 1999 Annual Data Report*, Bethesda, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 1999
 22. Kronenberg F, Utermann G, Dieplinger H: Lipoprotein(a) in renal disease. *Am J Kidney Dis* 27: 1–25, 1996
 23. Luc G, Bard JM, Arveiler D, Ferrieres J, Evans A, Amouyel P, Fruchart JC, Ducimetiere P: Lipoprotein (a) as a predictor of coronary heart disease: The PRIME Study. *Atherosclerosis* 163: 377–384, 2002
 24. Cantin B, Despres JP, Lamarche B, Moorjani S, Lupien PJ, Bogaty P, Bergeron J, Dagenais GR: Association of fibrinogen and lipoprotein(a) as a coronary heart disease risk factor in men (The Quebec Cardiovascular Study). *Am J Cardiol* 89: 662–666, 2002
 25. Marcovina SM, Koschinsky ML: Lipoprotein(a) as a risk factor for coronary artery disease. *Am J Cardiol* 82: 57U–66U, 1998
 26. Koschinsky ML, Marcovina SM: Lipoprotein(a): structural implications for pathophysiology. *Int J Clin Lab Res* 27: 14–23, 1997
 27. Edelberg JM, Gonzalez-Gronow M, Pizzo SV: Lipoprotein a inhibits streptokinase-mediated activation of human plasminogen. *Biochemistry* 28: 2370–2374, 1989
 28. Hervio L, Girard-Globa A, Durlach V, Angles-Cano E: The antifibrinolytic effect of lipoprotein(a) in heterozygous subjects is modulated by the relative concentration of each of the apolipoprotein(a) isoforms and their affinity for fibrin. *Eur J Clin Invest* 26: 411–417, 1996
 29. Marcovina SM, Koschinsky ML, Albers JJ, Skarlatos S: Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein(a) and Cardiovascular Disease: Recent advances and future directions. *Clin Chem* 49: 1785–1796, 2003