Increased Serum Lipoprotein(a) Concentrations and Low Molecular Weight Phenotypes of Apolipoprotein(a) Are Associated with Symptomatic Peripheral Arterial Disease

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Background: Increased concentrations of lipoprotein(a) [Lp(a)] have been considered a genetically determined risk factor for coronary artery and cerebrovascular disease. Only 2 small and conflicting studies have investigated the possibility of an association of peripheral arterial disease (PAD) with high serum Lp(a) concentrations and low molecular weight (LMW) phenotypes of apolipoprotein(a) [apo(a)].

Methods: We measured serum concentrations of Lp(a) and apo(a) phenotypes in 213 patients with symptomatic PAD and 213 controls matched for sex, age (within 2 years), and presence of diabetes.

Results: Patients with PAD showed significantly higher median serum concentrations of Lp(a) (76 vs 47 mg/L; P = 0.003) and a higher frequency of LMW apo(a) phenotypes (41% vs 26%; P = 0.002) than controls. After adjustment for several potential confounders, increased Lp(a) concentrations (>195 mg/L, i.e., 75th percentile of the entire study sample) and LMW apo(a) phenotypes were significant predictors of PAD, with odds ratios of 3.73 (95% CI 2.08–6.67; P < 0.001) and 2.21 (95% CI 1.33–3.67; P = 0.002), respectively.

Conclusions: In this study sample, both increased serum concentrations of Lp(a) and the presence of LMW apo(a) phenotypes were associated with the presence of symptomatic PAD independent of traditional and nontraditional cardiovascular risk factors. Because PAD is considered an indicator of systemic atherosclerotic disease, our results suggest a possible role of Lp(a) as a genetically determined marker for systemic atherosclerosis.

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Lipoprotein(a) [Lp(a)] consists of a large LDL-like particle to which the specific glycoprotein apolipoprotein(a) [apo(a)] is covalently linked (1). Serum concentrations of Lp(a) are genetically determined to a large extent by the polymorphic apo(a) gene on chromosome 6q27 (2). The apo(a) kringle IV (K-IV) repeat polymorphism with >30 alleles generates apo(a) molecules ranging from 300 to 800 kDa, with an inverse relationship between apo(a) size and Lp(a) concentration (3).

The physiological and pathophysiological role of Lp(a) is not well understood, but detection of Lp(a) in the vessel wall at the site of vascular injury indicates atherogenic and thrombogenic properties (4). There is evidence that oxidized Lp(a) contributes to foam cell formation (5). Furthermore, apo(a) sharing high sequence homology with plasminogen (6) has been reported to attenuate fibrinolysis and promote coagulation (7, 8).

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Nonstandard abbreviations: Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); K-IV, Kringle IV; LMW, low molecular weight; CVD, cerebrovascular disease; PAD, peripheral arterial disease; LIPAD, Linz Peripheral Arterial Disease; CAD, coronary artery disease; HMW, high molecular weight; tHcy, total homocysteine; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.
Recently, it has been demonstrated in transgenic mice that oxidized phospholipids occur primarily within Lp(a) and not within LDL, suggesting that Lp(a), when present at low concentrations, might function as a scavenger absorbing oxidized lipids and thereby prevent the uptake of other oxidized lipoproteins, especially LDL, by the vessel wall (9). In contrast, when Lp(a) is increased owing to the expression of low molecular weight (LMW) apo(a) isoforms, it might become proatherogenic because of its high content of oxidized lipids. This concept has been elegantly supported by several clinical investigations showing a strong association between oxidized phospholipids, Lp(a), and cardiovascular disease (10, 11).

Numerous epidemiological studies have identified increased Lp(a) concentrations and the presence of LMW apo(a) isoforms as risk factors for coronary artery and cerebrovascular disease (CVD) (12–15). However, only limited data exist on Lp(a) concentrations and apo(a) isoform distribution in systemic atherosclerotic disease. Atherosclerotic peripheral arterial disease (PAD) is the prototype of systemic (chronic) atherosclerosis and is characterized by arterial stenoses and occlusions in the peripheral arterial bed of the lower limbs (16–18). Two case–control studies have reported conflicting results concerning Lp(a) concentrations and apo(a) isoforms and an association with PAD (19, 20). Both studies were performed in rather small patient samples, and the technique available at that time permitted only limited resolution of the apo(a) isoform.

To address these issues in a large case–control study, we tested the hypothesis that increased serum Lp(a) concentrations and LMW apo(a) phenotypes are associated with symptomatic PAD in the Linz Peripheral Arterial Disease (LIPAD) study (21, 22).

**Materials and Methods**

**STUDY SAMPLE**

The LIPAD study was designed to evaluate possible phenotypic and genotypic risk factors for atherosclerotic PAD. The study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki, and all study participants gave informed consent. The study objectives, recruitment procedures, and characteristics were described in detail previously (21, 22). Patients (n = 433) with symptomatic atherosclerotic PAD admitted to the hospital for inpatient diagnostics and treatment of chronic limb ischemia were enrolled in the LIPAD case–control study. Of these, frozen serum samples were available from only 213 patients; these patients were eligible for the present study. For each patient with PAD, an appropriate referent was recruited by the investigators and matched to the patients with PAD in a 1:1 design by sex, age (within 2 years), and diabetes. All study participants were evaluated for the presence of risk factors for atherosclerosis and comorbid conditions, as recommended by Rutherford et al. (23).

PAD was defined as chronic atherosclerotic disease of the lower extremities associated with typical symptoms, such as claudication or leg pain on exertion, rest pain, or minor or major tissue loss, and was verified by interview, physical examination, and Doppler segmental blood pressure measurement of the lower limbs, including continuous wave spectral analysis and resting ankle-brachial index measurements, as well as intraarterial aortofemoral angiography. Patients with PAD were included in this study on the basis of the final clinical diagnosis established by the attending vascular surgeons. All cases with acute ischemia (i.e., peripheral arterial thrombosis of a native artery, popliteal artery aneurysm, or acutely thrombosed peripheral bypass grafts) were excluded. Additional exclusion criteria were PAD attributable to nonatherosclerotic causes (cardioembolic disease, thromboangiitis obliterans, vasculitis, or congenital or metabolic vascular disease) and a history or presence of any malignancy.

Coronary artery disease (CAD) was defined as remote myocardial infarction by history, occult myocardial infarction by electrocardiography, previous coronary bypass surgery or percutaneous transluminal coronary angioplasty, and stable or unstable angina and acute coronary syndrome (cardiac troponin positive or negative). CVD was defined as ischemic transient or temporary stroke, ischemic completed stroke with permanent neurologic deficit, or acute ischemic stroke. Arterial hypertension, diabetes, and smoking were classified according to recommended standards (23). Control participants were free of prevalent atherosclerotic disease (i.e., CAD, CVD, or PAD). All controls had an ankle-brachial index ≥1.0, no pathologic pattern of pulse waves in lower limbs by continuous-wave spectral analysis, no stenosis of the internal carotid artery ≥50% by color duplex ultrasound scan, and no history or presence of any malignancy. By study design none of the control participants received lipid-lowering medication or took folate supplement or B vitamins.

We collected blood by venipuncture in Vacuette polyethylene terephthalate glycol clot activator tubes (Greiner Bio-One) after the patient had fasted overnight. We obtained serum samples by low-speed centrifugation and stored them at −80 °C until laboratory analysis.

**Lp(A) MEASUREMENTS AND APO(A) PHENOTYPING**

We measured Lp(a) concentrations in duplicate using a double-antibody ELISA as described (24). Briefly, we used an affinity-purified polyclonal apo(a) antibody as coating antibody and the horseradish peroxidase–conjugated monoclonal antibody 1A2 recognizing the K-IV-2 domain of apo(a) for detection (25). This ELISA has an intraassay CV of 1.94% and an interassay CV of 4.95% (24) and was used in several previous studies (11, 14, 24, 26, 27). The results obtained were highly correlated (r = 0.978; P < 0.001) with those of a turbidimetric Lp(a) assay provided by Denka Seiken Co. Ltd. (A. Lingenhel, F.
Kronenberg, unpublished data), which has been reported to achieve the best concordance with a reference assay known to measure Lp(a) independently of apo(a) size (28).

We determined apo(a) isoforms by SDS agarose gel electrophoresis under reducing conditions and immunodetection, followed by densitometric semiquantitative determinations as outlined (26). We stratified apo(a) phenotypes in 2 subgroups according to the molecular weight of the smaller apo(a) isoform. We defined the LMW apo(a) phenotype by the occurrence of at least 1 apo(a) isoform with 11 to 22 K-IV repeats, and the high molecular weight (HMW) apo(a) phenotype was considered when all isoforms had >22 K-IV repeats.

**FURTHER BIOCHEMICAL ANALYSES**

We analyzed glucose, fasting glucose, glycohemoglobin A\textsubscript{1c}, total cholesterol, triglycerides, apolipoprotein A-I, and apolipoprotein B with standard assays on a COBAS Integra analyzer (Roche Diagnostics). To measure HDL cholesterol and LDL cholesterol, we used quantitative electrophoresis with enzymatic staining (Helena Biosciences Europe). For this work, we corrected LDL cholesterol for the proportion of Lp(a) cholesterol by subtracting 45% of the measured Lp(a) mass as described (27). We performed total homocysteine (tHcy), folate, and vitamin B\textsubscript{12} assays on an AxSYM analyzer (Abbott Diagnostics). We measured the concentration of C-reactive protein (CRP) with a high-sensitivity assay (N High Sensitivity CRP) on a BN ProSpec analyzer (Dade Behring) using polystyrene particles coated with monoclonal mouse antibodies to CRP. We calculated estimated glomerular filtration rate (eGFR) as recently recommended (29).

**STATISTICAL METHODS**

We performed statistical analysis using SPSS version 13.0 (SPSS). Dichotomous data were given as absolute and relative numbers, and continuous variables were expressed as median (25th–75th percentiles) unless otherwise indicated. We calculated univariable comparisons of risk factors and other dichotomous variables between the 2 study groups with the Fisher exact test or the Mantel–Haenszel test, as appropriate, and continuous variables were evaluated with the nonparametric Mann–Whitney U-test (respectively P values were not adjusted for multiple comparisons and are therefore only descriptive). To determine whether Lp(a) serum concentrations and apo(a) phenotypes were predictors for PAD and to calculate multivariable odds ratios, we performed logistic regression analysis without variable selection technique (confounding risk factors were simultaneously included in the regression model). Dichotomous risk factors were coded with an indicator variable of 1 for having the condition and 0 for its absence. We tested for interaction of Lp(a) and apo(a) phenotypes with other variables using logistic regression models with PAD as an independent variable. Probabilities were 2-tailed, and P <0.05 was regarded as statistically significant.

**Results**

The study sample (n = 426) included 316 men and 110 women with a median age of 66 years. Patients were admitted because of mild to severe claudication or leg pain on exertion (Fontaine stage II; n = 179; 84%), ischemic rest pain (Fontaine stage III; n = 6; 3%), and minor or major tissue loss (Fontaine stage IV; n = 28; 13%). Of the 213 patients with PAD, 67 (32%) had concomitant CAD and 35 (16%) had CVD. Furthermore, 64 patients with PAD had ≥50% carotid stenosis. Another 19 patients with PAD were classified as also having stenosis ≤50%, because they had undergone previous carotid surgery to treat stenosis. Per definition, the 213 controls matched to the patients with PAD for sex, age (within 2 years), and diabetes did not have CAD, CVD, or an internal carotid stenosis ≥50%. However, many control participants (n = 179) exhibited carotid plaques as a sign of mild, but not clinically relevant, atherosclerosis.

The clinical and biochemical characteristics are summarized in Table 1. Of the patients with PAD, 32% were treated with lipid-lowering medication; ~90% of them received HMG-CoA reductase inhibitors known to have no influence on Lp(a) concentrations (30), and ~10% received fibrates. Median serum concentrations of Lp(a) were significantly higher in the patients with PAD than in the controls (76 mg/L vs 47 mg/L; P = 0.003; Table 2). Furthermore, the frequency of LMW apo(a) phenotypes was significantly higher in patients with PAD than in controls (41% vs 26%; P = 0.002; Table 2). The median Lp(a) concentration and the prevalence of the LMW apo(a) phenotype did not differ significantly between PAD patients with concomitant CAD and/or CVD (n = 88) vs patients without concomitant CAD and/or CVD (n = 125) [89 vs 69 mg/L; P = 0.623; and n = 40 (46%) vs n = 47 (38%), P = 0.157, respectively].

We performed an unadjusted logistic regression analysis using an incremental approach for Lp(a) in the first instance to assess the relationship of Lp(a) and the presence of PAD. In this analysis, a 100 mg/L increase of Lp(a) revealed an odds ratio of 1.10 (95% CI 1.03–1.18; P = 0.007) for PAD (Table 3). To further test for a linear relationship of Lp(a) and the presence of PAD, we divided our entire study sample (n = 426) according to Lp(a) quartiles. Using this approach, odds ratios for PAD were 1.01 (95% CI 0.64–1.88; P = 0.736), 1.02 (95% CI 0.59–1.75; P = 0.951), and 2.42 (95% CI 1.39–4.19; P = 0.002) in the 2nd, 3rd, and 4th quartiles of Lp(a) concentrations compared with the 1st quartile, demonstrating a threshold effect at the 195 mg/L cut point (Table 3). Accordingly, this Lp(a) cutoff value was used to dichotomize Lp(a) values in low vs increased Lp(a) serum concentrations for all further statistical analyses.

The results of logistic regression models analyzing the ability of increased Lp(a) concentrations and LMW apo(a)
phenotypes to predict PAD independently of possible confounding variables are provided in Table 4. In unadjusted analysis, both increased Lp(a) concentrations (>195 mg/L, i.e., 75th percentile of the entire study sample) and LMW apo(a) phenotype were significantly associated with symptomatic PAD (model 1). The magnitude of these associations remained similar after adjustment for the matched variables age, sex, and diabetes status (model 2), and became even stronger after including other vascular risk factors and potential confounders, displaying odds ratios for PAD of 3.73 (95% CI 2.08–6.67; P < 0.001) in increased Lp(a) concentrations and 2.21 (95% CI 1.33–3.67; P = 0.002) in LMW apo(a) phenotypes (model 4).

When dichotomized Lp(a) concentrations and LMW apo(a) phenotype were simultaneously entered in a multiple logistic regression model, an increased Lp(a) concentration but not the LMW apo(a) phenotype was a significant predictor of symptomatic PAD (model 5).

We further tested for possible interactions of Lp(a) concentrations and apo(a) phenotypes with other variables (age, sex, diabetes, current smoking, arterial hypertension, and LDL cholesterol) using logistic regression models with PAD as an independent variable. For these analyses, we used median values of the entire study sample for the continuous variables of interest (age, 66 years; LDL cholesterol, 1270 mg/L). No statistically sig-
significant interaction between increased Lp(a) (>195 mg/L) and LDL cholesterol (P for interaction 0.427) on PAD was observed in the entire study sample (n = 426). Furthermore, we did not detect a statistically significant interaction between the apo(a) phenotype and LDL cholesterol (P for interaction 0.386). Similarly, no significant interactions were present between Lp(a) or apo(a) phenotypes with the other variables mentioned above (data not shown).

To determine the combined effect of increased Lp(a) concentrations and LMW apo(a) phenotypes, we conducted a further adjusted logistic regression analysis in which the study participants were stratified into 4 groups according to Lp(a) concentrations and apo(a) phenotypes, as shown in Fig. 1. The odds ratio for PAD was highest among participants with increased Lp(a) concentrations and LMW apo(a) phenotype (odds ratio 4.62; 95% CI 2.36–9.04; P < 0.001). In contrast, we found no significant associations with PAD in individuals with either increased Lp(a) concentrations and HMW apo(a) phenotype (odds ratio 1.66; 95% CI 0.57–4.87; P = 0.536) or low Lp(a) concentrations and LMW apo(a) phenotype (odds ratio 0.92; 95% CI 0.44–1.92; P = 0.818).

**Discussion**

In this case–control study, we demonstrated that increased Lp(a) serum concentrations and LMW apo(a) phenotypes are associated with the presence of symptomatic PAD independent of traditional and nontraditional cardiovascular risk factors.

So far, only 2 studies with inconsistent results have investigated the role of Lp(a) concentrations and apo(a) isoforms in patients with symptomatic PAD (19, 20). In both studies, increased Lp(a) concentrations were associated with PAD. Only Mölgaard et al. (20) found a higher prevalence of LMW apo(a) phenotypes in patients with PAD than in the control group, whereas Pedro-Botet et al.

| Table 2. Lp(a) serum concentrations and apo(a) size polymorphism in patients with PAD and control participants. |
|----------------------------------|----------------------------------|-----------------|-----------------|
| **Lp(a), mg/L**                  | **PAD group (n = 213)**          | **Control group (n = 213)** |
| Median (25th–75th percentiles)  | 76 (24–311)                      | 47 (20–132)     |
| Mean (SD)                        | 227 (315)                        | 150 (255)       |
| Apo(a) alleles, n (%)^a          |                                  |                 |
| 11–19 K-IV repeats               | 36 (17%)                         | 16 (7%)         |
| 20–22 K-IV repeats               | 51 (24%)                         | 40 (19%)        |
| 23–25 K-IV repeats               | 25 (12%)                         | 35 (17%)        |
| 26–28 K-IV repeats               | 25 (12%)                         | 30 (14%)        |
| 29–31 K-IV repeats               | 26 (12%)                         | 42 (20%)        |
| >31 K-IV repeats                 | 50 (23%)                         | 50 (23%)        |
| Apo(a) phenotypes, n (%)^b       |                                  |                 |
| LMW apo(a) phenotypes            | 87 (41%)                         | 56 (26%)        |
| HMW apo(a) phenotypes            | 126 (59%)                        | 157 (74%)       |

^a Mann–Whitney U-test for comparison of Lp(a) serum concentrations between patients and controls; P = 0.003.

^b Mantel–Haenszel test for linear association comparing the frequencies of apo(a) alleles between patients and controls; P = 0.016.

^c Fisher exact test comparing the frequencies of apo(a) phenotypes between patients and controls; P = 0.002.

| Table 4. Results of logistic regression models analyzing the ability of increased Lp(a) concentrations and LMW apo(a) phenotypes to predict PAD independently of possible confounding variables. |
|----------------------------------|----------------------------------|-----------------|-----------------|
| Logistic regression models analyzing the ability of increased Lp(a) concentrations (i.e., >195 mg/L) to predict PAD |
| Model 1^a                        | 2.33 (1.48–3.68)                 | <0.001          |
| Model 2^a                        | 2.33 (1.48–3.68)                 | <0.001          |
| Model 3^a                        | 2.73 (1.63–4.57)                 | <0.001          |
| Model 4^a                        | 3.73 (2.08–6.67)                 | <0.001          |
| Model 5^a                        | 3.27 (1.66–6.44)                 | 0.006          |

^d Odds ratios were calculated by multiple logistic regression analysis without variable selection technique (all independent variables were simultaneously included in the model).

^e Not adjusted for possible confounders.

^f Adjusted for age, sex, and diabetes status.

^g Adjusted for age, sex, diabetes status, body mass index, current smoking, and arterial hypertension.

^h Adjusted for age, sex, diabetes status, body mass index, current smoking, arterial hypertension, eGFR, HDL cholesterol, LDL cholesterol, triglycerides, hsCRP, and tHcy.

^i Adjusted for age, sex, diabetes status, body mass index, current smoking, arterial hypertension, eGFR, HDL cholesterol, LDL cholesterol, triglycerides, hsCRP, tHcy, and LMW apo(a) phenotype.

^j Adjusted for age, sex, diabetes status, body mass index, current smoking, arterial hypertension, eGFR, HDL cholesterol, LDL cholesterol, triglycerides, hsCRP, tHcy, and Lp(a) concentration >195 mg/L.

| Table 3. Results of univariable logistic regression models analyzing the ability of Lp(a) concentrations to predict PAD. |
|----------------------------------|----------------------------------|-----------------|-----------------|
| Logistic regression analysis using an incremental approach for Lp(a) concentrations |
| Lp(a) (+ 100 mg/L)               | 1.10 (1.03–1.18)                 | 0.007           |
| Logistic regression analyses according to the quartiles of Lp(a) concentrations |
| 1st quartile (<22 mg/L, n = 107) | 1.0                              |                 |
| 2nd quartile (22–60 mg/L, n = 106)| 1.01 (0.64–1.88)               | 0.736           |
| 3rd quartile (60–195 mg/L, n = 106)| 1.02 (0.59–1.75)            | 0.951           |
| 4th quartile (>195 mg/L, n = 107)| 2.42 (1.39–4.19)               | 0.002           |
(19) found no difference in apo(a) phenotype distribution between cases and controls. Both studies were performed in rather small sample populations containing only male samples [Mølgaard et al. (20), n = 100; Pedro-Botet et al. (19), n = 89]. Because Lp(a) serum concentrations vary over a 1000-fold range and show a highly skewed distribution (3), results of small studies should be considered with caution (31). Furthermore, the respective phenotypes were designated according to the nomenclature of Utermann et al. (3), who were the first to identify 6 apo(a) isoforms by polyacrylamide gel electrophoresis. However, technical refinements, in particular the introduction of high-resolution SDS agarose gel electrophoresis by Kamboh et al. (32), which was later refined by Marcovina et al. (33), have made it feasible to distinguish more than 30 isoforms at present. Consequently, there was a need to further elucidate the role of Lp(a) and apo(a) isoforms in PAD with enhanced sample size and refined apo(a) phenotyping methods that allow the detection of even low expressed apo(a) isoforms.

Our study found increased Lp(a) serum concentrations (>195 mg/L, 75th percentile) and the LMW apo(a) phenotype to be strongly associated with symptomatic PAD. Of note, the observed association was independent of other confounders. In addition, a secondary analysis revealed that there was no significant difference in Lp(a) serum concentrations and apo(a) isoforms between PAD patients with and without concomitant clinically manifest CAD and/or CVD, indicating independence of our findings from the presence of clinically manifest atherosclerosis in other vascular territories. This observation is therefore in line with the results of a recent case–control study by Jones et al. (34), who demonstrated that an increased Lp(a) concentration is a stable risk factor of similar magnitude for PAD, CAD, and CVD within the same study population. It is, however, impossible to completely disentangle PAD (as a marker of systemic atherosclerotic disease) from CAD and CVD because CAD and CVD have been demonstrated to be prevalent in up to 90% of patients with PAD, depending on the detection technique used (35). This holds true in particular for early atherosclerotic disease in the coronary vasculature.

Interestingly, the apo(a) size polymorphism was no longer a significant predictor of PAD as soon as Lp(a) concentrations were entered in the same logistic regression model. This is explained by the fact that (depending on the ethnic origin of the population investigated) the apo(a) K-IV repeat polymorphism explains only ~15% to 70% of the Lp(a) variance in addition to other genetic variability within the apo(a) gene (36). Therefore, Lp(a) concentration was a stronger predictor of symptomatic PAD than was the K-IV repeat polymorphism.

It has been proposed that increased Lp(a) concentrations and LMW apo(a) phenotypes may synergistically contribute to Lp(a) pathogenicity (37). In the Bruneck study, increased Lp(a) concentrations—when associated with LMW apo(a) phenotypes—emerged as a stronger predictor of advanced stenotic carotid atherosclerosis than each risk factor alone. In contrast, in the same study, for early carotid atherosclerosis only Lp(a) concentrations, but not apo(a) phenotypes, were found to be predictive (14). Conversely, the Physician’s Health Study demonstrated that small apo(a) size was an independent predictor of angina pectoris, regardless of Lp(a) concentration, and that no additive effect or synergism was present (13). Hence, conflicting reports exist on the relative role of Lp(a) concentrations and apo(a) isoforms in coronary disease and CVD. We found that the synergistic effect of increased Lp(a) concentrations and the LMW apo(a) phenotype revealed the strongest association with symptomatic PAD, suggesting that the increased Lp(a) concentrations in patients with PAD are genetically determined by the higher frequency of LMW apo(a) isoforms.

Of note, in our study we did not find an interaction of LDL cholesterol with Lp(a) concentrations or apo(a) phenotypes on their associations with symptomatic PAD. These findings are in accordance with the results of the Bruneck study (14) demonstrating that the relationship between high Lp(a) concentrations and advanced stenotic carotid atherosclerosis was not modified by high LDL cholesterol concentrations. Conversely, in the same study a significant association of Lp(a) concentrations and early carotid atherosclerosis was confined to those with increased LDL cholesterol (14), and the Physician’s Health Study as well as the Women’s Health Study provided evidence that Lp(a) and LDL cholesterol concentrations synergistically interact to increase cardiovascular risk.
(13, 15). Taken together, data available on the interaction of Lp(a) and LDL cholesterol in early vs advanced atherosclerosis might indicate a different impact of LDL cholesterol concentrations on the atherogenic properties of Lp(a) depending on the magnitude of disease burden. Thus, the interaction between Lp(a) and LDL cholesterol described for early atherosclerotic disease may not hold true for advanced atherosclerotic disease (such as symptomatic PAD).

Our study clearly demonstrates the relationship between increased Lp(a) concentrations and LMW apo(a) phenotypes with symptomatic PAD. Because of the strong association of apo(a) phenotypes with Lp(a) concentrations, a measurement of Lp(a) without detection of apo(a) phenotypes might be sufficient for risk stratification. This is different from the case in patients with end-stage renal disease, who can best be risk-stratified by the apo(a) polymorphism (38, 39), which can be explained by the apo(a) isoform-dependent increase in Lp(a) concentration (26, 40).

Potential limitations of our study warrant consideration. The present study is a post hoc analysis of our previously described study cohort. Unfortunately, frozen serum samples were available for only 213 of the 433 patients originally enrolled in the LIPAD case–control study (21, 22). Furthermore, our study population of patients with symptomatic PAD admitted to the hospital who were probably severely diseased patients with advanced systemic atherosclerosis (17) may have strengthened the effect size. Therefore, further studies are needed to elucidate the role of Lp(a) and apo(a) isoforms as risk factors for asymptomatic PAD.

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