

Plasma Lipoprotein(a) Is an Independent Factor Associated With Carotid Wall Thickening in Severely but Not Moderately Hypercholesterolemic Patients

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Background and Purpose To evaluate whether high levels of low-density lipoprotein cholesterol (LDL-C) may promote the atherogenic effect of lipoprotein(a) [Lp(a)], we investigated the association between elevated Lp(a) levels and thickening of intima plus media in the common carotid artery (CC-IMT) in patients with different degrees of hypercholesterolemia.

Methods One hundred type II hypercholesterolemic patients and 25 normolipidemic subjects were selected for the study. Plasma lipid and lipoprotein levels were determined enzymatically; Lp(a) levels were determined by enzyme-linked immunosorbent assay. An Lp(a) concentration >30 mg/dL was arbitrarily considered a risk factor. For each patient mean CC-IMT was determined by B-mode ultrasound; in 60 patients and in the 25 control subjects, the maximal IMT in the entire carotid tree was also determined.

Results CC-IMT values were higher in hypercholesterolemic patients with plasma Lp(a) levels >30 mg/dL than in those with lower levels ($P < .01$). CC-IMT and maximal IMT

directly and independently correlated with plasma levels of Lp(a) ($r = .33$ and $r = .25$, respectively; both $P < .05$). The effect of LDL-C concentrations on the relationship between IMT and Lp(a) was investigated by dividing the patients into quartiles of plasma LDL-C levels. After stratification, CC-IMT significantly correlated with plasma Lp(a) levels in the patients with severe hypercholesterolemia (LDL-C >5.2 mmol/L) but not in patients in the lowest quartile, ie, those with moderate hypercholesterolemia. No correlation between CC-IMT and Lp(a) was found in normolipidemic control subjects.

Conclusions Elevated plasma levels of Lp(a) can be considered an additional independent factor associated with thickening of the common carotid arteries in patients with severe hypercholesterolemia but not in those with moderate hypercholesterolemia or in normocholesterolemic subjects. (*Stroke*. 1996;27:1044-1049.)

Key Words • atherosclerosis • lipoproteins, LDL cholesterol • carotid arteries

A number of case-control and prospective studies have demonstrated a strong association between elevated plasma Lp(a) levels and symptomatic coronary or extracoronary vascular disease and suggest that high Lp(a) is an independent risk factor for atherothrombotic disease.¹ However, high plasma Lp(a) is frequently associated with elevated concentrations of LDL-C,² which are themselves atherogenic. In particular, patients with familial hypercholesterolemia have high Lp(a) levels, and this raises the question of the clinical significance of Lp(a) in high-risk patients. In addition, the relative risk for myocardial infarction is significantly higher in subjects with high plasma Lp(a) levels when LDL-C concentrations are also elevated,²⁻⁵ so that the clinical relevance of Lp(a) may be different in normolipidemic and hypercholesterolemic subjects.

High-resolution B-mode ultrasonography allows the accurate assessment of the presence and extent of carotid atherosclerosis.⁶⁻⁸ CC-IMT determined by this technique has been used to investigate the impact of risk factors,

including cigarette smoking, male sex, age, hypertension, diabetes mellitus, fibrinogen, and hypercholesterolemia, on early asymptomatic atherosclerosis.^{7,9-13}

An association between elevated plasma Lp(a) levels and echographically detected carotid atherosclerosis has been found in patients with cerebrovascular disease¹⁴ as well as in asymptomatic individuals.^{15,16} Little is known, however, about the relationship between elevated Lp(a) and early atherosclerosis in hypercholesterolemic patients.^{17,18} In particular, no information is available on the relationship between Lp(a) and carotid atherosclerosis measured by arterial wall thickness. We have examined Lp(a) as a risk factor for intima-media carotid thickening in hypercholesterolemic patients and also whether the effect of plasma Lp(a) levels on carotid wall thickening depends on the LDL-C concentration.

Subjects and Methods

Hypercholesterolemic patients (49 men and 51 women, aged 20 to 71 years) with a diagnosis of type II hyperlipoproteinemia by the criteria of the World Health Organization¹⁹ were selected from consecutive patients entering the E. Grossi Paoletti Center for the Study of Metabolic Disorders. Those with established atherosclerotic lesions, as defined by a personal history of angina, claudication, or cerebrovascular ischemia, were excluded. Heavy smokers were also excluded; in the entire group there were 6 smokers (<10 cigarettes per day). The selected patients had no clinical signs of arterial occlusive disease, eg, bruits or decreased vascular pulses. In addition,

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Selected Abbreviations and Acronyms

CC-IMT	= common carotid intima-media thickness
HDL-C	= high-density lipoprotein cholesterol
LDL-C	= low-density lipoprotein cholesterol
Ln-Lp(a)	= log-transformed lipoprotein(a)
Lp(a)	= lipoprotein(a)
Max-IMT	= maximal intima-media thickness
TC	= total cholesterol
TG	= triglyceride(s)

none had hypertension or diabetes mellitus. At the time of blood sampling, the subjects were on a diet free of restrictions and had stopped any lipid-lowering treatments for at least 2 months.

Twenty-five normocholesterolemic subjects, otherwise similar in clinical status and age, constituted a control group. All normolipidemic subjects were recruited among the staff of the center. Oral informed consent was obtained from patients and control subjects.

Fasting venous blood was collected in Na₂-EDTA (1 mg/mL). Plasma TC and TG were determined by enzymatic methods²⁰; HDL-C was determined after selective precipitation of apoprotein B-containing lipoproteins with dextran sulfate/MgCl₂.²¹ Plasma LDL-C was calculated by Friedewald's formula.²² The individual LDL-C value was corrected for the Lp(a)-cholesterol content by subtracting 0.3 times the corresponding plasma [Lp(a)] on the assumption that Lp(a) contains 30% cholesterol.²³ Plasma Lp(a) was determined by enzyme-linked immunosorbent assay (MACRA, Terumo) with the use of solid-phase-bound monoclonal and peroxidase-labeled polyclonal antibodies against apoprotein(a).²³ The linearity and accuracy of the Lp(a) assay were repeatedly verified; interassay and intra-assay coefficients of variation were 9.8% and 6.7%, respectively.

Carotid ultrasound imaging was performed with an echotomographic system (model 2000 II, Biosound), with a probe that generates a wide-band ultrasound pulse with a midfrequency of 8 MHz. The axial and lateral resolutions are approximately 0.385 and 0.500 mm, respectively. With this technique, two parallel echogenic lines separated by an anechoic space can be visualized at the arterial wall.⁶ These lines have been shown to be generated by the blood-intima and media-adventitia interfaces.^{6,7} The distance between the lines is a reliable index of the thickness of the intima-media complex.⁶

Extracranial common carotid arteries of the neck were scanned in three different projections (anterior, lateral, and posterior) by a single operator who was blinded with respect to the clinical characteristics of the patients. Briefly, each common carotid artery was examined in the craniocaudal direction starting from the crest of the bifurcation. Images obtained from each were recorded on VHS videotapes. The main source of variability in the evaluation of CC-IMT was previously found to lie in the operator's subjectivity in the choice of carotid segments to be processed.²⁴ To avoid image overlapping, maximal care was undertaken in probe placement along the common carotid artery.

In 60 patients and in the 25 control subjects, the arterial walls (far and near) of the bulb and the internal and external carotid arteries were also examined in anterior, lateral, and posterior planes, with the crest of the bifurcation and the flow divider serving as points of reference. Single maximal localized plaques were measured, and this Max-IMT was also considered in evaluating the relationship of Lp(a) to carotid atherosclerosis. Age, plasma lipids, and blood pressure of the 40 patients without a Max-IMT determination were not different from those of the remaining 60 patients with complete data (data not shown).

Images of carotid arteries were obtained by "freezing" videotape frames on a television monitor. Printouts of selected frames were obtained with a video copy processor (model 70 B, Mitsubishi).

The echographic device visualizes anatomic structures in a field approximately 2 cm long; therefore, to obtain a complete picture of each common carotid artery we produced two to four carotid segments for each projection, depending on the length of the artery, and the mean CC-IMT of the sectors was calculated (see below). For measurement of common carotid segments, we determined the area (A) between the luminal and deeper echoes using the graphic tablet of a personal computer.⁷ The precise length of each segment was measured with the same device and identified as L1, L2, etc. All measurements were performed under blind conditions. The mean CC-IMT was calculated for each common carotid artery in each patient according to the following formula:

$$\text{CC-IMT} = (A1 + A2 \dots + An) / (L1 + L2 \dots + Ln)$$

Values for the different projections and for right and left arteries were then averaged.

Accuracy and reproducibility for mean CC-IMT were 4.6% and 5.0%, respectively.^{7,24} The reproducibility of the CC-IMT measurement was determined on two scans performed by the same sonographer (D.B.) on 14 subjects 2 weeks apart. The videotapes were read and CC-IMT was measured by another observer. The correlation between CC-IMT in the first and second scans was .94. The relative difference between the first and second measurements ranged from -0.14 to 0.18 mm, with a mean of 0.023 mm and SD of 0.088 mm.

Mean and SD values were used as descriptive measures of normally distributed variables; medians and ranges were used in the case of non-normally distributed variables. Because of their highly skewed distribution, TG and Lp(a) values were log transformed to yield an almost gaussian distribution. Comparisons of mean CC-IMT values of subgroups were performed by nonpaired Student's *t* test. Differences in categorical variables were analyzed by the χ^2 test. Correlations were performed by parametric methods (Pearson correlation). Multiple stepwise regression analysis was applied to assess the relative importance of the independent variables. All statistical analyses were performed with the use of Statgraphics software (STSC, Inc. and Statistical Graphic Corp).

Results

Table 1 shows the characteristics of the hypercholesterolemic patients enrolled in the study. To examine the influence of plasma Lp(a) levels on carotid atherosclerosis, we chose a cutoff point of 30 mg/dL on the basis of studies that have concluded that plasma Lp(a) values of 30 to 40 mg/dL constitute a coronary risk.^{18,25,26} Patients were thus arbitrarily divided into group A [plasma Lp(a) \leq 30 mg/dL] and group B [plasma Lp(a) > 30 mg/dL]. The groups differed significantly in plasma TG (lower in group B) and TC and LDL-C levels (lower in group A). Mean CC-IMT values in group B were significantly higher than those in group A (Table 1).

Correlation analysis on the entire series of hypercholesterolemic patients ($n=100$) showed that plasma Lp(a) (Fig 1, left panel) and age (Fig 1, right panel) both positively correlated with CC-IMT values ($r=.33$ and $r=.22$, respectively; $P<.05$). The correlation between CC-IMT and plasma Lp(a) persisted after stratification by age (age < 50, \geq 50 to \leq 60, and > 60 years) (data not shown), which indicates that age and Lp(a) do not interact in determining carotid thickening. Indeed, by stepwise multiple regression analysis, plasma Lp(a) and age were significant independent predictors of CC-IMT, accounting for 15% of the variability of CC-IMT (Table 2).

No difference in Ln-Lp(a) levels was observed between men (2.84 ± 1.31 mg/dL) and women (2.83 ± 1.28

TABLE 1. Characteristics of Hypercholesterolemic Patients and Differences in CC-IMT Between Hypercholesterolemic Groups

	All Hypercholesterolemic Patients	Hypercholesterolemic Patients	
		Group A [Lp(a) ≤30 mg/dL]	Group B [Lp(a) >30 mg/dL]
Lp(a), mg/dL	19.5 (1-165)	8.0 (1-29.5)	67.5 (30.3-165)*
n	100	60	40
Women	51	31	20
Men	49	29	20
Age, y	55.2±9.4	54.9±7.9	55.4±11.1
TC, mmol/L	8.18±1.87	7.89±1.67	8.62±2.05†
TG, mmol/L	1.51 (0.58-4.81)	1.62 (0.58-4.81)	1.24 (0.79-4.34)†
HDL-C, mmol/L	1.28±0.39	1.27±0.35	1.28±0.43
LDL-C, mmol/L	6.05±1.95	5.66±1.8	6.64±2.01†
TC/HDL-C	6.82±2.15	6.55±1.91	7.2±2.41
Plasma glucose, mmol/L	5.27±0.65	5.37±0.69	5.13±0.55
BMI, kg/m ²	23.5±3.6	23.9±3.9	22.7±2.9
DBP, mm Hg	84.6±7.1	83.6±8.1	85.1±7.1
SBP, mm Hg	130.6±12.3	131.0±12.1	132±11.6
CC-IMT, mm	0.66±0.17	0.62±0.16	0.72±0.16‡

BMI indicates body mass index; DBP, diastolic blood pressure; and SBP, systolic blood pressure. Results are expressed as mean±SD except for Lp(a) and TG, which are expressed as median and range. Statistical significance was determined by unpaired Student's *t* test for all variables except TG and Lp(a), which were analyzed by the Mann-Whitney *U* test.

*Difference results from selection of studied group.

†*P*<.05, ‡*P*<.01, group A vs group B.

mg/dL). CC-IMT significantly correlated with plasma Lp(a) levels in both men ($r=.38$; $P=.006$) and women ($r=.27$; $P=.04$).

The effect of LDL-C as an independent predictor of CC-IMT became evident only after correction of individual LDL-C values for the Lp(a)-cholesterol content. Indeed, when the corrected LDL-C was considered in the stepwise multiple regression analysis, age, corrected LDL-C, and Ln-Lp(a) proved to be significant independent predictors of CC-IMT, together accounting for 25% of the variability of CC-IMT (Table 3).

To investigate whether the relationship between Lp(a) and CC-IMT is affected by the individual plasma LDL-C level, statistical analysis was performed after stratification of hypercholesterolemic patients into quartiles according to the level of LDL-C (Table 4). The significant correlation between CC-IMT and Lp(a) observed in the total group of hypercholesterolemic patients was present only in the patients of the upper three quartiles of LDL-C. In addition, there was no correlation between CC-IMT and Lp(a) in a group of 25 normocholesterolemic subjects (in whom CC-IMT positively correlated with age and LDL-C level). These subjects (16 men, 9 women; age, 27 to 63 years; diastolic blood pressure, 82.6±9.5 mm Hg; systolic blood pressure, 134.2±9.9 mm Hg) had the same characteristics of the patients, except

for lower TC ($5.3±0.8$ mmol/L), LDL-C ($3.3±0.8$ mmol/L), and mean CC-IMT ($0.53±0.09$ mm).

After stratification of subjects into severe (LDL-C >5.2 mmol/L, $n=75$) and normal plus moderately hypercholesterolemic (LDL-C ≤5.2 mmol/L, $n=50$) groups, stepwise multiple regression analysis identified Ln-Lp(a) ($R^2=.12$, $P<.002$) as the only predictor of CC-IMT in severely hypercholesterolemic patients and identified age ($R^2=.33$, $P<.0001$) as the only predictor of CC-IMT in moderately hypercholesterolemic patients. The same results were obtained when we used corrected LDL-C.

To investigate whether elevated Lp(a) affects not only average wall thickening along the common carotid artery but also the occurrence of localized atherosclerotic lesions in the entire carotid tree, we investigated the relationship between plasma Lp(a) levels and the Max-IMT of the entire carotid tree. Max-IMT correlated significantly with CC-IMT ($r=.53$, $P<.001$; Fig 2), confirming that CC-IMT is a reliable index of atherosclerosis along the entire carotid tree.

A significant correlation between Max-IMT and Lp(a) was found in hypercholesterolemic patients ($r=.25$, $P<.05$) but not in control subjects ($r=.12$, $P=NS$). As for

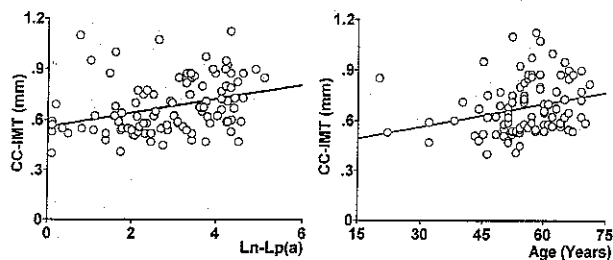


FIG 1. Correlation between CC-IMT and Ln-Lp(a) (left) and age (right) in hypercholesterolemic patients.

TABLE 2. Stepwise Multiple Regression Analysis in Hypercholesterolemic Patients (CC-IMT as Dependent Variable)

Variable	b	SE (b)	T	P*
Ln-Lp(a)	.0368	.012	3.11	.002
Age	.0037	.001	2.32	.022
Multiple R=.364				
SE=.150				
$R^2=.156$				
F=8.9 ($P=.0003$)				

Variables not in equation: TC, log TG, HDL-C, LDL-C, TC/HDL-C, glucose, and body mass index.

*For T values.

TABLE 3. Stepwise Multiple Regression Analysis With cLDL-C Instead of LDL-C in Hypercholesterolemic Patients (CC-IMT as Dependent Variable)

Variable	b	SE (b)	T	P*
Ln-Lp(a)	.02	.009	2.15	.033
cLDL-C	.0004	.0002	2.25	.025
Age	.006	.001	5.06	.00001

Multiple R=.51

SE=.14

R²=.25

F=10.54 (P=2.34×10⁻⁷)

cLDL-C indicates LDL-C corrected for Lp(a)-cholesterol content. Variables not in equation: TC, log TG, HDL-C, TC/HDL-C, glucose, and body mass index.

*For T values.

CC-IMT, after stratification of subjects into severe and moderate hypercholesterolemia groups, stepwise multiple regression analysis identified as independent predictors of Max-IMT the Ln-Lp(a) in severely hypercholesterolemic patients (R²=.1, P<.001) and age and LDL-C in moderately hypercholesterolemic patients (R²=.34, both P<.05). Aside from the threshold effect, LDL-C levels again did not significantly affect the association of Lp(a) with Max-IMT in hypercholesterolemic patients.

Discussion

In this study the impact of elevated plasma LDL-C levels on the association between Lp(a) and extracranial carotid atherosclerosis in asymptomatic hypercholesterolemic patients was investigated. Lp(a) was found to be a risk factor for intima-media carotid thickening and for the occurrence of localized atherosclerotic lesions, but only in patients with severe hypercholesterolemia (LDL-C >5.2 mmol/L). This finding is consistent with previous reports of an interaction between Lp(a) and LDL-C in determining the risk for coronary heart disease.²⁻⁵ However, while for coronary disease an additive or synergistic interaction between LDL-C and Lp(a) has been suggested, the present results argue for a threshold effect of LDL-C, with no further interaction between Lp(a) and LDL-C in determining carotid wall thickening.

CC-IMT was on average 16% thicker in hypercholesterolemic patients with plasma Lp(a) levels >30 mg/dL than in those with Lp(a) levels ≤30 mg/dL. However, the finding of a direct correlation between CC-IMT and Lp(a) not only strengthens the association between these two variables observed in the univariate analysis but also indicates that Lp(a) should be considered a continuous

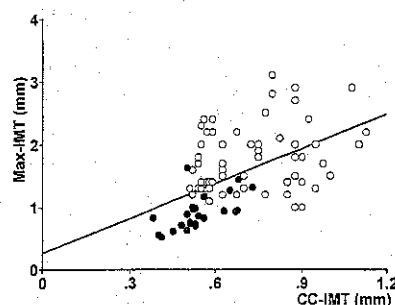


Fig 2. Correlation between CC-IMT and measurements of a single localized plaque (Max-IMT). The correlation was calculated with the use of pooled data from 25 normolipidemic control subjects (●) and 60 hypercholesterolemic patients (○).

variable that influences carotid thickening. Correlation analysis indicated that CC-IMT also correlated directly with age. The independent influence of the aforementioned variables on carotid wall thickness was further confirmed by multiple stepwise regression analysis.

Interestingly, CC-IMT correlated highly with Max-IMT, a variable more widely accepted than CC-IMT as a surrogate index of carotid atherosclerosis. Moreover, LDL-C and Lp(a) showed the same pattern of correlation when either CC-IMT or Max-IMT was used.

A direct association between plasma Lp(a) levels and the degree of carotid atherosclerosis assessed by B-mode ultrasonography has been widely reported.^{14-18,27} Two of these studies,^{17,18} which also examined hypercholesterolemic patients, showed a positive association between Lp(a) and carotid atherosclerosis by grading the carotid lesions either as carotid score¹⁸ or dichotomous variables (presence/absence of plaque).¹⁷ A more sensitive method for the evaluation of carotid atherosclerosis was followed in the present study, in which CC-IMT, a reliable index of carotid²⁸ and possibly also coronary atherosclerosis,^{29,30} was taken into account.

High Lp(a) concentrations have been reported to increase the relative risk for carotid atherosclerosis only in the presence of high plasma LDL-C levels.¹⁷ In particular, a significant association between "carotid plaques" and elevated Lp(a) was found in patients with severe hypercholesterolemia (>4.7 mmol/L) but not in those with moderate hypercholesterolemia (LDL-C ≤4.7 mmol/L).¹⁷ This finding is confirmed by our observation of a direct correlation between CC-IMT and Lp(a) only in patients with markedly elevated plasma LDL-C levels (LDL-C >5.2 mmol/L). Such correlation

TABLE 4. Correlations Between CC-IMT and Lp(a), Lipids, and Age in Normocholesterolemic and Hypercholesterolemic Subjects After Stratification by Quartiles of LDL-C

	CC-IMT				
	Normocholesterolemic Subjects	Hypercholesterolemic Subjects (by Quartiles of LDL-C)			
		1.4-3.5	4.1-5.2	>5.2-5.9	>5.9-6.8
Ln-Lp(a)	-.21	-.05	.39*	.23*	.47*
TC	.31	-.24	-.32	.22	.06
LDL-C	.38*	-.17	-.12	-.08	.09
Age	.80†	.46*	.07	.3	.23

LDL-C values are expressed as limits of quartiles in millimoles per liter.

*P<.05, †P<.001.

was not found in patients with moderate hypercholesterolemia or in normolipidemic subjects.

In the present study LDL-C was measured by Friedewald's formula and was possibly overestimated because of the presence of a small percentage of cholesterol attributable to Lp(a).²³ Data analysis in which a corrected value was used did not modify the results and confirmed both LDL-C and Lp(a) as independent predictors of CC-IMT.

Age was the second variable associated with CC-IMT in the entire group of hypercholesterolemic patients. After stratification for LDL-C, age was strongly associated with CC-IMT only in patients with moderate hypercholesterolemia (LDL-C \leq 5.2 mmol/L) as well as in control subjects. The lack of age-related CC-IMT increases in patients with severe hypercholesterolemia (LDL-C $>$ 5.2 mmol/L) suggests that the effect of LDL and/or Lp(a) outweighs the effect of age. When patients were stratified by age, the correlation between Lp(a) and CC-IMT was still present in all age classes, indicating that age does not significantly affect the association between Lp(a) and common carotid wall thickening.

Several studies suggest the existence of a role for sex hormones in modulating Lp(a) concentrations,³¹⁻³⁴ but this relationship has not been confirmed by others.³⁵⁻³⁷ In our group of hypercholesterolemic patients, men and women were equally represented. However, no difference in plasma Lp(a) levels was observed between men and women, and the correlation between Lp(a) and CC-IMT, observed in the entire hypercholesterolemic group, although stronger in men was evident also in women.

A growing body of data shows that CC-IMT is affected by major atherosclerosis risk factors^{7,9-13} and is highly correlated with the occurrence of plaques in the entire carotid tree,¹⁰ but there is still debate on whether the CC-IMT is an index of early atherosclerosis or a consequence of nonatherosclerotic processes such as medial hyperplasia. The observation of a highly significant correlation between CC-IMT and Max-IMT and of a similar effect of Lp(a) on CC-IMT and Max-IMT provides further support for the concept that CC-IMT is a reliable index of carotid atherosclerosis.

The mechanism(s) by which Lp(a) acts at the vascular level have not yet been elucidated. Lp(a) may influence plasminogen activation by reducing local plasmin generation.³⁸ Indeed, in mice transgenic for human apolipoprotein(a), an impaired plasmin generation affects the activation of transforming growth factor- β , with proatherogenic consequences.³⁹ Lp(a) may also affect the rate of lipid deposition in the atheromatous plaque,^{26,40} thus playing an atherogenic role in the arterial wall. These mechanisms provide biological plausibility to the present observation of a direct relationship between elevated plasma Lp(a) levels and the extent of carotid atherosclerosis.

The observation that Lp(a) is positively correlated with carotid atherosclerosis only in the presence of elevated levels of LDL is difficult to explain. The quantitative nature of the CC-IMT measurement rules out the possibility that this finding derives from methodological problems in the echographic examination. It is well known that the relative risk of myocardial infarction increases significantly in subjects with high Lp(a) levels when concentrations of LDL are also high,^{2,41} which suggests an interaction between these two factors. It is

important to emphasize that LDLs are themselves potent atherogenic factors that induce carotid thickening.^{7,10} It can thus be hypothesized that Lp(a) is more atherogenic in conditions of vascular wall perturbation. This hypothesis, however, needs to be tested in appropriate in vitro and/or in vivo models.

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