Research Article

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Biomarkers in Medicine

Association between Lp (a) and atherosclerosis in menopausal women without metabolic syndrome

Aim: The association between Lipoprotein (a) (Lp [a]) and common carotid intima media thickness (IMT) has been evaluated in 222 menopausal women. **Material & methods:** Lp (a) and IMT were measured, carotid ultrasound examination (B-Mode imaging) was performed and mean max IMT was calculated. **Results:** Lp (a) was significantly lower in women with metabolic syndrome (MS). In a multivariate analysis Lp (a) showed the following odds ratio (OR; all $p < 0.05$) of having common carotid IMT (≥1.30 mm): 1.03, adjusted for age, low-density lipoprotein cholesterol (LDL) and waist circumference; 1.02, adjusted for age LDL, homeostatic assessment model (HOMA). In women without MS, after controlling for age, LDL and waist circumference, we found the following OR for increased IMT (≥1.30; OR: 1.03; for Lp [a]); 1.02 adjusted for age, LDL and HOMA (all $p < 0.05$). In women with MS these relationships were not statistically significant. **Conclusion:** Lp (a) gives additional information in the risk assessment for atherosclerotic cardiovascular disease, especially in menopausal women without MS.

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Lp (a) is a lipoprotein which is similar, but metabolically different from low-density lipoproteins (LDL), and its structure contains Apolipoprotein (a) (apo[a]), the size of which is highly variable and genetically determined [1]. Lp (a) is an atherogenic lipoprotein [2]. Elevated plasma concentrations of Lp (a) are an independent, causal risk factor for coronary heart disease, in particular of myocardial infarction [3], stroke [4] and peripheral arterial disease [5]. In a prospective meta-analysis [6], the risk ratio for Cardiovascular disease, corresponding to 3.5-fold higher Lp (a) levels is 1.16 (95% CI: 1.11–1.22).

High-resolution B-mode ultrasound has been used for the noninvasive assessment of intima media thickness (IMT), a marker of early atherosclerosis which predicts cardiovascular events [7]. Cardiovascular risk factors relate positively to IMT of the carotid artery [8].

In the present analysis, we evaluated if Lp (a) was associated with early carotid atherosclerosis in a sample of menopausal women, participating in a large, ongoing, population-based, prospective study ('Progetto ATENA') [9].

Methods

Study population

The Progetto Atena was carried out on 5062 women aged 30–69 years living in the area of Naples (southern Italy) [9]. This study was focused on investigation of chronic diseases in adult women. At baseline visit, none of the participants had previous diagnoses of cardiovascular disease or cancer. As a part of the design, a recall of 228 women – randomized among the older ones – was scheduled, after

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Table 1. Clinical and biochemical characteristics of women, categorized by the metabolic syndrome diagnosis.

Variable	No (n = 128)	Yes (n = 94)
Age (years)	62.5 ± 8.7	64.1 ± 7.4
Triglycerides (mg/dL)†	89.7 ± 31.5	137.5 ± 68.1**
High-density lipoprotein cholesterol (mg/dL)	61.9 ± 11.5	52.0 ± 11.5**
Fasting glucose (mg/dL)	98.1 ± 15.9	115.6 ± 31.9**
Systolic blood pressure (mmHg)	137.7 ± 19.7	150.5 ± 19.2**
Diastolic blood pressure (mmHg)	79.7 ± 8.3	84.1 ± 7.2**
Waist circumference (cm)	85.9 ± 8.9	98.6 ± 15.9**
Total cholesterol (mg/dL)	220.1 ± 36.1	230.0 ± 40.5*
Low-density lipoprotein cholesterol (mg/dL)	140.2 ± 33.1	150.5 ± 34.3*
Apo B (g/L)	1.0 ± 0.2	1.1 ± 0.2**
High sensitive CRP (mg/L)†	2.3 ± 2.6	3.3 ± 5.6*
Insulin (mU/L)	5.4 ± 2.7	8.4 ± 5.3**
Body mass index (kg/m^2)	26.2 ± 3.9	30.5 ± 4.1**
Homeostatic assessment model index (HOMA)†	1.3 ± 0.7	2.4 ± 1.5**
Lp (a) (mg/dL)†	27.9 ± 29.7	19.1 ± 22.1*
Intima Media Thickness (mm)	1.04 ± 0.2	1.06 ± 0.2*

Values are shown as mean ± standard deviation.
*p < 0.05.
**p < 0.001.
†U-Mann–Whitney.

10 years from the first visit. On these participants, all of them in post-menopause status, defined as the loss of menstrual bleeding for 1 year [10], a number of biological and biochemical investigations were performed, including Lp (a) determinations. No significant differences in cardiovascular risk profile between the subsample and the remaining cohort (n = 4834) were detected [11]. All participants signed an informed consent, and the protocol of the study was approved by the ethics board of the 'Federico II' University (Naples, Italy).

High resolution carotid ultrasound

The same carotid B-mode ultrasound protocol was performed for each subject by an experienced sonographer, certified for quantitative reading of ultrasound records using a ESAOTE AU4 (ESAOTE SPA, France). First, IMT was measured in the distal 1.0 cm of the near and far walls of the common carotid artery. The crest at the origin of the bifurcation was used as an anatomical landmark to identify this segment. This analysis was repeated for the left common carotid artery. In each carotid ultrasound imaging, different scanning angles were examined (anterior, lateral and posterior) to allow the identification of the greatest IMT in each wall. Scans were recorded for offline analysis [12]. Readers carefully reviewed the carotid ultrasound scans and selected the frame that contained the thickest IMT for each of the four carotid walls. The mean of these four maximum thicknesses was used as the ultrasound end point of the study. Common carotid IMT measurements were available for all 228 subjects. Quality control data, derived from a large international multicenter trial, demonstrated a coefficient of reliability for the common carotid IMT of 0.85 [13]. The IMT end point for this analysis was IMT ≥1.30, which was interpreted as early subclinical atherosclerosis (carotid thickening or plaque), and corresponds to the 90th percentile for IMT in these post-menopausal women (mean age 63.1 years).

Clinical & biochemical assessment

According to the American Heart Association statement [14], and with the exclusion of patients on lipid-lowering drugs, metabolic syndrome (MS) was diagnosed when three or more of the following criteria were present:

- Elevated waist circumference (WC): ≥102 cm in men, ≥88 cm in women;
- Elevated triglycerides: ≥150 mg/dL; reduced high-density lipoprotein cholesterol: <40 mg/dL in men, <50 mg/dL in women;

- Elevated blood pressure: systolic ≥130 mmHg or diastolic ≥85 mmHg; drug treatment of hypertension;

- Elevated fasting glucose: ≥100 mg/dL; drug treatment of diabetes.

BMI, a measure of overweight/obesity, was calculated as weight (kg) divided by height (in m^2). WC, an index of abdominal obesity, was measured midway between the bottom of the rib cage and the top of the iliac crest. Blood pressure was measured by a random zero sphygmomanometer. A standard questionnaire was used to collect information about smoking habits. Blood was drawn by puncture of antecubital vein after a 12-h fast.

Total cholesterol, triglyceride and high-density lipoprotein-cholesterol concentrations were measured using enzymatic methods [11]. LDL cholesterol was calculated according to the Friedewald formula. Fasting glucose was determined enzymatically by the peroxidase method. Fasting insulin levels were determined by enzyme immunoassay (Ultrasensitive Insulin Elisa, Mercodia, Sweden). The error of the method (between-run coefficient of variation [CV]) was <10%. Apo B and hs-CRP were measured with turbidimetric assay with an automated method (Cobas-Mira, Roche, Italy). The error of the method (between-run CV) was <5%. Lp (a) was measured by an ELISA, solid phase two-site enzyme immunoassay, using polyclonal antibodies raised against purified Lp (a) (Mercodia Diagnostics, Uppsala, Sweden) [15]. The error of the method was <10%. Homeostatic assessment model (HOMA) index was used to assess insulin resistance and calculated as fasting serum insulin (μU/ml) × fasting serum glucose (mM)/22.5, as described by Matthews *et al.* [16]. All biochemical analyses were carried out on fresh blood sample. Only Lp (a) determinations were done on frozen samples (-80°C). Samples for Lp (a) measurement were not available for six women.

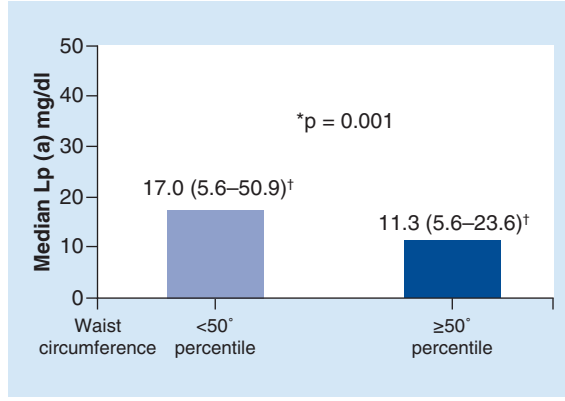

Figure 1. Lp (a) by waist circumference values (n = 222).
*U-Mann-Whitney.
†Medians (interquartile ranges).

Statistical analyses

Statistical analyses were carried out using SPSS version 13.0 (SPSS, Inc., IL, USA). Continuous variables were described as mean and standard deviation or standard error.

Comparisons between women with and without the MS were performed using Student's t-test for inde-

Table 2. Relationships of intima media thickness ≥1.3 mm, Lp (a) with other variables (multivariate logistic analysis; n = 222).

Independent variables	Dependent variable IMT†		
	p-value	OR	95% CI for OR lower – upper
Model I			
Age years‡	<0.001	1.15	1.07–1.24
Lp (a) mg/dL‡	0.003	1.03	1.01–1.05
LDL mg/dL‡	0.021	1.01	1.00–1.03
WC cm‡	0.016	1.07	1.01–1.13
Model II			
Age years‡	<0.001	1.15	1.00–1.03
Lp (a) mg/dL‡	0.019	1.02	1.01–1.05
LDL mg/dL‡	0.012	1.01	1.00–1.03
HOMA Units‡	0.264	1.24	0.85–1.8

†Discrete variable with IMT ≥1.3 mm as outcome variable.
‡Continuous variables.
HOMA: Homeostatic assessment model; IMT: Intima media thickness; LDL: Low-density lipoprotein-cholesterol; OR: Odds ratio; WC: Waist circumference.

Table 3. Relationships of intima media thickness ≥1.3 mm, Lp (a) with other variables in women without metabolic syndrome: multivariate logistic analysis (n = 128).

Independent variables	Dependent variable IMT†		
	p-value	OR	95.0% CI for OR lower – upper
Model I			
Age years ‡	0.002	1.19	1.06–1.33
Lp (a) mg/dL‡	0.006	1.03	1.01–1.06
LDL mg/dL‡	0.091	1.02	0.99–1.04
WC cm‡	0.015	1.11	1.02–1.21
Model II			
Age years‡	0.003	1.18	1.06–1.33
Lp (a) mg/dL‡	0.021	1.02	1.00–1.05
LDL mg/dL‡	0.074	1.02	0.99–1.04
HOMA Units‡	0.183	1.58	0.80–3.1

†Discrete variable with IMT ≥1.3 mm as outcome variable.
‡Continuous variables.
IMT: Intima media thickness; LDL: Low-density lipoprotein cholesterol; OR: Odds ratio; WC: Waist circumference.

pendent-samples and nonparametric test (U-Mann–Whitney test), for variables (CRP, triglycerides and Lp [a]) with skewed distribution. Correlation coefficients, nonparametric Spearman's rho were used to test correlations between Lp (a) and other biochemical variables.

Univariate and multivariate logistic regression analysis were performed to calculate odds ratio for IMT (independent variable). Multivariate logistic regression analysis was performed to test the independent relation between age, WC, insulin, HOMA, LDL-cholesterol (independent variables) and IMT (≥1.3 mm). Odds ratio (OR) for one unit increase of age, Lp (a), LDL-Cholesterol, WC, HOMA was calculated by unconditional logistic regression and 95% CI of the odds ratio were computed.

Results

Out of the 222 participants, 94 (42.3%) had metabolic syndrome (MS), 7(32.9%) took antihypertensive drugs, 25 (11.3%) took hypocholesterolemic drugs, 56 (25.2%) were smokers, 71 (32.0%) were moderate drinkers and 23 (10.4%) had diagnosis of diabetes. Of these women, 70.9% were overweight/obese.

Clinical and biochemical characteristics of the study participants categorized by MS are shown in Table 1. In addition to expected differences in MS components (Table 1), women with the MS diagnosis had higher total Cholesterol, LDL-Cholesterol, Apo B, Hs-CRP, Insulin, BMI, HOMA and IMT as compared with participants without MS.

On the other hand, Lp (a) was significantly lower in women with MS (19.1 mg/dL vs 27.9 mg/dL, p < 0.05 by U-Mann–Whitney). Lp (a) was negatively correlated (Spearman's rho) with WC (p = 0.023), insulin (p < 0.001) and HOMA (p < 0.001); in addition, Lp (a) was positively correlated with total cholesterol (p = 0.023) and LDL-cholesterol (p = 0.022).

Women with lower WC (<50° percentile) had higher Lp (a) compared with women with elevated WC (≥50° percentile; median 17.0 vs 11.3; p < 0.001 by Mann–Whitney) (Figure 1). In addition, women with lower HOMA (<50° percentile) had a higher Lp (a) as compared with woman with elevated HOMA (≥50° percentile; median 17.0 vs 11.3; p < 0.001 by Mann–Whitney test).

In a univariate logistic analysis, Lp (a) was associated with increased IMT (≥1.30; OR: 1.02; p = 0.005). We then evaluated, in a multivariate logistic analysis, the association between high common carotid IMT (≥1.30 mm) and Lp (a), taking into account different adjustment models (Table 2). Lp (a) showed the following OR for high common carotid IMT (≥1.30 mm): 1.03 (p = 0.003), adjusted for age, LDL, WC; 1.02 (p = 0.019), adjusted for age, LDL and HOMA. In a subsequent multivariate analysis, we evaluated the relation between IMT and Lp (a), in women without metabolic syndrome (Table 3). After controlling for age, LDL and WC, women without metabolic syndrome (n = 128) showed the following OR for increased IMT (≥1.30; OR: 1.03; p = 0.006 for Lp [a]) and after controlling for age, LDL and HOMA OR (1.02; p = 0.021 for Lp [a]). Women with MS (n = 94), after controlling for age, LDL and WC, showed the following (not significant) OR for increased IMT (≥1.30; OR: 1.08; p = 0.129 for Lp [a]) or after controlling for

age, LDL and HOMA, OR: 1.13; p = 0.640 for Lp (a).

Discussion

We demonstrate an association between Lp (a) and early atherosclerosis in menopausal women. The association is detectable only in women without MS. This association is independent of age, LDL cholesterol, WC and HOMA in a multivariate analysis. To our knowledge no study is available on the relationship between Lp (a) and IMT in menopausal women, from southern Italy. A limitation of this study is the cross-sectional design, however we can detect a significant association between Lp (a) and carotid IMT in menopausal women.

A number of studies investigated the relation between MS and Lp (a). A study [17] carried out on an Asian occupational cohort showed an inverse association between Lp (a) concentrations and MS and its components. High levels of Lp (a) were associated with preclinical atherosclerosis, as evaluated by coronary calcium score. In a recent study by Marzano *et al.* [18], it was demonstrated that insulin resistance (HOMA) and higher fasting insulin levels were associated with lower plasma Lp (a), in nondiabetic, middle-aged hypertensive patients (but in this study IMT was not evaluated). An elegant *in vitro* study by Neele *et al.* [19] demonstrated that increasing insulin concentrations induced a suppression of Apo (a) synthesis in primary cultures of monkey hepatocytes: this mechanism could explain the inverse relationship between plasma Lp (a) and insulin, in our study and previous ones [17,18].

Our survey in healthy menopausal women, participating in a large population-based cohort (Progetto Atena) study, demonstrated that the association of Lp (a) with relatively higher carotid IMT was independent of age, LDL and markers of MS.

Our data indicate that the association between Lp (a) and increased carotid IMT was present in healthy menopausal women without metabolic syndrome, while the association was not detectable in women with MS. We suggest that the presence of MS is a main confounder, when evaluating the relationship between Lp (a) and early atherosclerosis, in menopausal women, who often show a high prevalence of MS.

Carotid wall thickening and plaques are predictors of future cardiovascular events [7,20]. Elevated plasma concentrations of Lp (a) are an independent, causal risk factor for coronary heart disease [21]. Lp (a) measurement gives additional information on the risk for early atherosclerotic cardiovascular disease, especially in menopausal women without metabolic syndrome, by identifying a subgroup with high Lp (a) at higher risk for cardiovascular events.

Conclusion

Lp (a) measurement gives additional information on the risk for early atherosclerotic cardiovascular disease, especially in menopausal women without metabolic syndrome, by identifying a subgroup with high Lp (a) at higher risk for cardiovascular events.

Future perspective

This paper provides useful data on menopausal women, especially without metabolic syndrome, by identifying a subgroup with high Lp (a) at higher risk for cardiovascular events.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Association between Lp (a) and Intima Media Thickness is independent of age, low-density lipoproteins, waist circumference and homeostatic assessment model in menopausal women without metabolic syndrome.
- Lp (a) gives additional information on the risk for atherosclerotic cardiovascular disease in menopausal women, especially without metabolic syndrome.

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