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Lipoprotein (a) and 10-year cardiovascular disease incidence in apparently healthy individuals: a sex-based sensitivity analysis from ATTICA cohort study

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Abstract
The association between lipoprotein (a) (Lp(a)) and 10-year first fatal/non-fatal cardiovascular disease (CVD) risk in apparently healthy men and women was evaluated. The ATTICA prospective study was conducted during 2001-2012 and included *n*=1,514 men and *n*=1,528 women (aged >18 years old) from the greater Athens area, Greece. Follow-up CVD assessment (2011-2012) was achieved in *n*=2,020 participants (*n*=317 cases); baseline Lp(a) was measured in *n*=1,890 participants. The recommended threshold of 50 mg/dL was used to define abnormal Lp(a) status. Ten-year CVD-event rate was 14% and 24% in participants with Lp(a) <50 and Lp(a) ≥50 mg/dL, respectively. Multivariate analysis revealed that participants with Lp(a) ≥50 mg/dL *vs* Lp(a) < 50 mg/dL had about 2 times higher CVD risk (Hazard Ratio (HR)=2.18, 95% Confidence Interval (95%CI) 1.11, 4.28). Sex-based analysis revealed that the independent Lp(a)-effect was retained only in men (HR=2.00, 95%CI 1.19, 2.56); in women, significance was lost after adjusting for lipid markers. Sensitivity analyses revealed that Lp(a) increased CVD risk only in case of abnormal high density lipoprotein cholesterol, apolipoprotein A1 and triglycerides as well as low adherence to Mediterranean diet. Certain patient characteristics may be relevant when considering Lp(a) as a therapeutic or risk-prediction target.
Key words: lipoprotein (a); lipid markers; sex; cardiovascular disease; heart disease; primary prevention

Introduction

The existing risk prediction models that include conventional risk factors present a satisfactory discriminative ability for cardiovascular disease (CVD) onset in both men and women.[1] Even if this seems to leave comparatively little potential for emerging risk factors, there is increasing interest in novel biomarkers in an effort to identify their contribution to risk stratification, improve the understanding of pathophysiological mechanisms and help design new treatment approaches.[2] Such markers include alternative lipids, B-type natriuretic peptides, high-sensitivity troponin, coronary artery calcium, and genetic markers.[2]. Considering the sex differences in the pathophysiology of CVD, the predictive role of novel CVD biomarkers should be considered separately in men and women.[3] The literature provides limited evidence towards this sex-based

perspective.[4] Despite the increasing referencing to this topic, mainly within the last 5 years, there is room for improvement.[5]

Among novel CVD risk factors, lipoprotein (a) (Lp(a)) is a highly discussed biomarker with promising evidence regarding its predictive as well as prognostic value.[6] This "risk-factor" hypothesis is supported by the accumulation of its Lp(a) particles in human atherosclerotic lesions, the findings of Mendelian randomization studies and the amplification of plaque area in animal models expressing apolipoprotein (a).[7-8] Current metrics suggest that Lp(a) affects cardiac health in 1 out of 3 individuals, globally.[9] Furthermore, despite the emerging hypothesis that Lp(a) confers a more pronounced CVD risk in women, there remains controversy about the existence of a sex-related shape of the Lp(a) risk curve.[10] Recent data from 3 large-scale cohorts suggest that Lp(a) may have an independent aggravating effect only in females with hypercholesterolemia.[11] In contrast to this work, the investigators from the JUPITER clinical trial highlighted a strong association of Lp(a) with CVD even when total cholesterol levels were within the normal range, but only for men.[11]

The primary purpose of the present study was to evaluate the association between Lp(a) levels and 10-year first fatal/non-fatal CVD risk, the potential mediating effect of sex on the such an association as well as the contribution of Lp(a) to the predictive ability of an epidemiological model with traditional CVD risk factors. The secondary purpose was to assess the results from sensitivity analyses based on lipidemic profile and dietary habits of the participants. We posed 3 *a priori* research hypotheses: Lp(a) will be independently associated with 10-year combined CVD risk; Lp(a) will interact with sex regarding CVD onset; the contribution of Lp(a) to CVD risk stratification will be significant.

Materials/Subjects and Methods

Study sample

The ATTICA study is a prospective, observational cohort investigation which was initiated in 2001.[12] At baseline (2001-2002), *n*=3,042 apparently healthy volunteers residing in the greater metropolitan Athens area, Greece, agreed to participate (75% participation rate). Of the enrolled participants, *n*=1,514 (49.8%) were men (46±13 years) and *n*=1,528 (50.2%) were women (45±14 years). During baseline examination, a detailed clinical evaluation was performed by trained physicians; all participants were free of CVD and other chronic diseases, according to the study protocol. For the scope of the present work, we initially used the *n*=2,020 participants with complete CVD evaluation in the follow-up assessment. Then, for our primary analysis, we excluded *n*=130 participants with missing Lp(a) and other lipid marker [i.e. total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and their apolipoproteins (apolipoprotein B100 (ApoB) and apolipoprotein A1 (ApoA1), triglycerides] values; the final sample was *n*=1,890.

Bioethics

The ATTICA study was approved by the Bioethics Committee of Athens Medical School. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association. All participants were informed about the study aims and procedures and provided written informed consent.

Lp(a) and other lipid markers measurements at baseline examination

Blood samples were collected from an antecubital vein, between 8 to 10 a.m., in a sitting position after 12h fasting and alcohol avoidance. Serum for blood lipid measurements was prepared immediately after collection. The biochemical evaluation was carried out in a laboratory that followed the criteria of the World Health Organization Lipid Reference Laboratories. Serum total cholesterol, HDL-C and triglycerides were measured using chromatographic enzymic method using a Technicon automatic analyzer RA-1000 (Dade Behring, Marburg, Germany). HDL-C was determined after precipitation of the ApoB-containing lipoproteins with dextran-magnesium-chloride. Lp(a) was measured by a latex enhanced turbidimetric immuno-assay. The biochemical evaluation was carried out in the same laboratory that followed the criteria of the World Health Organization Lipid Reference Laboratories. The clinically recommended – by the European Society of Atherosclerosis – threshold of 50 mg/dL was used to define Lp(a) status as normal i.e. Lp(a) <50 mg/dL *vs* abnormal Lp(a) ≥50 mg/dL [1]; in our sample, this cut-off point had the best discriminative ability against the outcome of interest i.e. 10-year CVD event, through the receiver operating curve (ROC) analysis and the obtained – separately for men and women – optimal point. Besides this, the cut-off point of 30 mg/dL suggested in the literature, was also examined.[9] LDL-C (mg/dL) was calculated using the Friedewald formula:

(total cholesterol) – (HDL-C) – 1/5*(triglycerides) (only for participants with triglycerides <400 mg/dL). ApoB and apoA1 were measured by rate immunonephelometry. Lp(a)-corrected LDL-C, that is not taken into account by the Friedewald equation, was derived using the formula Lp(a)-cholesterol=Lp(a) mass * 0.3. An internal quality control was in place for assessing the validity of cholesterol, triglycerides and HDL-C methods. The intra and inter-assay coefficients of variation of cholesterol levels did not exceed 9%, triglycerides 4%, HDL-C 4% and Lp(a) 4%. Cut-off values of the aforementioned lipid markers were defined according to the most updated guidelines for dylipidaemias.[1]

Other baseline measurements

The assessed sociodemographic and lifestyle characteristics included age, sex, body mass index, level of adherence to Mediterranean diet, smoking habits and biomarkers related with renal function, liver function and systemic inflammation. Height was measured to the nearest 0.5 cm, with participants not wearing shoes, their backs square against the measuring wall tape, eyes looking straight ahead, with a right-angled triangle resting on the scalp and against the wall. Weight was measured with a balance, to the nearest 100 g, without shoes and in light undergarments. Body mass index was calculated as weight (in kg) divided by height (in m^2). Dietary habits were evaluated through a semi-quantitative food-frequency questionnaire (FFQ), originally developed for the European Prospective Investigation into Cancer and Nutrition study and provided by the Unit of Nutrition of Athens Medical School in its Greek version.[13] Level of adherence to Mediterranean diet was evaluated through the MedDietScore (range 0-55).[14-15] In the present work, the median MedDietScore value was used as cut-off point; in particular, low adherence to Mediterranean diet was defined as MedDietScore <27 and was assessed against moderate to high adherence to this dietary pattern i.e. MedDietScore ≥27). Regarding biochemical measurements, C-reactive protein (CRP; mg/L) were assayed by particle-enhanced immunonephelometry and used to define low grade inflammation. Creatinine was measured in serum, using a colorimetric method (BioAssay Systems, Hayward, CA). Renal function was evaluated according to Creatinine clearance ($C_{(Cr)}$). Based on serum creatinine measurements, the $C_{(Cr)}$ was calculated using the Cockcroft and Gault formula: $C_{(Cr)}$=[(140 − age) × weight]/(72*serum creatinine)] for men, whereas for women, the result of the above equation was multiplied by 0.85. Aminotransferases (i.e. alanine transaminase and aspartate transaminase) were measured using a chromatographic enzymic method and an automatic analyzer (RA-1000, Dade Behring, Marburg, Germany). The intra-assay and inter-assay coefficient of variation was <5%.

Further details regarding the methods and measurements applied have been previously described.[12, 16-17]

Endpoint and follow-up evaluation

Ten-year follow-up of ATTICA study was performed in 2012. The combined endpoint studied in this work was the development of a fatal or non-fatal CVD event. A CVD event was defined as acute myocardial infarction, or unstable angina, or other identified forms of ischemia (WHO-ICD coding 410-414.9, 427.2, 427.6), or heart failure of different types and chronic arrhythmias (WHO-ICD coding 400.0-404.9, 427.0-427.5, 427.9) or stroke (WHO-ICD coding 430-438). For participants who died during follow-up, information was retrieved from relatives and death certificates.

Statistical analysis

Categorical variables are presented as absolute (*n*) and relative frequencies (%). Continuous variables are presented as mean values ± standard deviation or median (Interquartile Range) if normality was not met. Associations between normally distributed variables and Lp(a) status were evaluated through Student's t-test for independent samples. Whether these variables were normally distributed was tested through P-P plot and equality of variances through Levene's test. For non-normally distributed variables, the Mann-Whitney test was used. Associations between categorical variables and Lp(a) status were tested with the chi-squared test. Hazard Ratios (HR) and their corresponding 95% Confidence Intervals (95%CI) for the Lp(a) status in relation to 10-year CVD event were evaluated through multivariable Cox-regression analysis in the total sample, as well as in subgroups. Proportional hazards' assumption was graphically tested. Total or CVD-case related correct classification rate was also obtained from the aforementioned multivariate analyses. Interactions between groups of participants were tested, and when significant, analyses were further stratified. The concordance statistics i.e. C-statistics was used to evaluate the predictive accuracy of multivariate models adjusted for various lipid markers against 10-year CVD event. C-indexes and the corresponding 95%CIs were equal to the areas under the curve obtained from the ROC analysis. Curves were constructed by plotting

sensitivity against 1-specifity. The STATA software, version 14 (MP & Associates, Sparta, Greece) was used for all statistical analyses. Two sided level of significance was set at $p<0.05$.

Results

At baseline, the mean Lp(a) value in the total sample of the ATTICA study was 19±23 mg/dL (17±21 mg/dL in men and 21±24 mg/dL in women, *p=0.02*). The prevalence of Lp(a) values over the clinically recommended threshold of 50 mg/dL was 8.5% (7.5% in men *vs* 9.5% in women, *p=0.21*). 19.2% of men and 19.2% women exceeded the threshold of 30 mg/dL. As mentioned before, for the purposes of the present analysis, only *n*=1,890 participants with complete CVD evaluation metrics at 10-year follow-up were retained for further analyses. In this subsample, the 10-year CVD event rate was 15.4% (*n*=291) [19.5% (*n*=184) in men and 11.3% (*n*=107) in women, *p<0.001*].

Baseline sociodemographic and clinical characteristics of participants as well as their metrics for biochemical markers according to Lp(a) status are summarized in **Table 1**. The 10-year CVD event rate across the Lp(a) categories is also presented in **Table 1**. Unadjusted models revealed that participants with Lp(a) over the generally accepted clinical value presented about 1.6 times higher CVD event rate compared with their counterparts with Lp(a) within the normal range (<50 mg/dL; *p=0.05*).

[Table 1]

Nested Cox regression models to evaluate the association between Lp(a) categories and 10-year CVD event are presented in **Table 2** (Lp(a) cut-off point of 50 mg/dL) **and Table 3** (Lp(a) cut-off point of 30 mg/dL). Starting with Table 2, in the unadjusted model, participants with Lp(a) ≥50 mg/dL presented almost 2.65 times higher risk for developing 10-year CVD events, compared with their lower Lp(a) values counterparts (*p<0.001*). Multiadjusted models where demographic and clinical factors were taken into account revealed that Lp(a) ≥50 mg/dL retained its significantly aggravating effect (*Model 2 and 3*). In *Model 4* the association was controlled for other lipid markers as well as for the use of statins; even in this case participants with Lp(a) ≥50 mg/dL presented about 2 times higher risk for incident CVD within the 10-year follow-up period (*p<0.05*). In the fully adjusted model where participants' kidney and liver function as well as systemic inflammation were included in the analysis the level of significance was retained (*Model 5*, *p<0.05*). The same multi-adjusted models were performed in case of the Lp(a) threshold of 30 mg/dL, yet no significant outcomes were observed as presented in **Table 3**.

[Table 2]

[Table 3]

Considering the hypotheses regarding the potential differences on the association between Lp(a) and incident CVD between men and women, among the aims of the present work was to evaluate the interacting effect of sex on the examined association as well as the sex-based effect size of Lp(a)-status (i.e. 50 mg/dL threshold) aggravating effect on the risk to develop a cardiac episode within the decade. A significant interaction was observed between sex and Lp(a) status on 10-year CVD event (*p for interaction=0.01*). Hence, nested Cox regression models were developed separately for men and women and results are shown in **Figure 1**. Age-standardized models showed that the male:female hazard rate ratio was 1.24 revealing that a male with abnormal Lp(a) status is at higher risk to develop a cardiac episode within the decade compared with a female at the same age and Lp(a) status (*Model 2*). In the subgroup of men, further adjustment with clinical factors, lipid markers as well as biomarkers related with systemic inflammation, liver and kidney function resulted in a progressively reduction of the effect size of the examined association yet without losing its significance (*Model 5*, *p<0.05*). On the other hand, in case of women, when conventional lipid markers were taken into account i.e. Lp(a)-corrected LDL-C, HDL-C, triglycerides as well as the statin use, the independent effect of Lp(a) status was lost; in the fully adjusted model i.e. *Model 5*, Lp(a) ≥50 mg/dL retained its aggravating effect on 10-year CVD incidence, yet without reaching the level of significance (*p>0.05*). The Lp(a) cut-off point of 30 mg/dL was also examined separately for men and women in relation to 10-year CVD event, yet no significant outcomes were reached, in line with the total-sample analysis presented in Table 3 (data not presented on table).

[Figure 1]

Due to the observed interacting effect of sex on the association between Lp(a) status and 10-year CVD risk, the discrimination ability of multivariate epidemiological models adjusted for Lp(a) and other lipid

markers was evaluated separately for men and women and results are presented in **Table 4**. Overall, the discrimination ability (expressed through C-index) of the examined multiadjusted models was better in case of women. However, the level to which Lp(a) contributed to the discrimination ability of the model was higher in the subgroup of men; in particular the difference between the estimated C-index in the model adjusted for both conventional lipid markers and Lp(a) and the C-index corresponding to the model adjusted only for conventional lipid markers was >0.01. This was not observed in case of women. The total correct classification rate was higher in case of *Model 1* (i.e. adjusted only for conventional lipid markers) in women (89.6%) with the correct classification rate for CVD cases being more than twice as high in *Model 1 vs Model 2* (adjusted only for Lp(a)) (19.6 *vs* 8.5%). As for the subgroup of men, the Lp(a)-adjusted model presented about 4 times higher correct classification rate for CVD cases (24.8%) compared with the respective number in women while their fully adjusted model (i.e. *Model 3*, all lipid markers) corresponded to the highest total as well as case-related correct classification rate.
[Table 4]

A formal analysis of interaction was also performed. Patients' lipidemic status in terms of total cholesterol seemed to interact on the examined association (*p for interaction<0.05*). Hence, it was decided to perform an extensive sensitivity analysis within the different clusters of lipidemic profile from the standpoint of total cholesterol, LDL-C, HDL-C and their apolipoproteins as well as the level of triglycerides. Additionally, formal analysis of interaction revealed that the level of adherence to Mediterranean diet had a significant interacting effect on the examined association. Results from stratified analyses with the Lp(a) status defined through the cut-off point of 50 mg/dL and the aforementioned markers as strata are presented in **Table 5**. As it was revealed, from the clusters of lipidemic profile only Lp(a)-corrected total cholesterol, HDL-C, ApoA1 and triglycerides presented a significant interacting effect on the examined association (*all p values for interaction <0.05*); to this effect, the independent association between Lp(a) status and 10-year CVD event was retained only in participants with hypercholesterolemia accompanied by abnormal HDL-C, ApoA1 and triglycerides levels (*all p values <0.05*). As for the level of adherence to Mediterranean diet, only in the context of MedDietScore below the median value participants with Lp(a) ≥50 mg/dL had an independently increased CVD risk within the decade compared with their counterparts with lower Lp(a) values (*p=0.02*); in participants with moderate to high level of adherence to this dietary pattern, the level of significance was lost. Further interaction analysis taking into account the sex, a triple significant interaction between sex, Lp(a) and specific lipid markers i.e. HDL-C, ApoA1 and triglycerides was observed (*all p values for interaction <0.05*); however additional stratification was not performed for statistical power limitations. The triple interaction between sex, MedDietScore and Lp(a) was also examined yet this did not reach the level of significance (*p for interaction=0.52*). In all the aforementioned cases, the analyses were re-performed with the Lp(a) status being defined in terms of 30 mg/dL as cut-off point; no significant outcomes were observed (data not presented on table).
[Table 5]

Discussion

In the present work, the independently aggravating effect of Lp(a) on incident CVD was confirmed with the risk increasing in values above the generally accepted cut-off point of 50 mg/dL.[1] In accordance with the recent literature, this observation is differentiated between sexes. In particular, our findings suggest that the role of Lp(a) on cardiac health may be more important for men, probably presenting higher predictive ability, yet more complex in case of women. Sensitivity analyses revealed that the independent aggravating role of Lp(a) was more evident in the context of total cholesterol, HDL-C, ApoA1 and triglycerides above the normal range as well as in participants with low adherence to Mediterranean diet; further interaction analysis suggested that some of these observations, mainly from the standpoint of lipidemic profile, could be sex-mediated. Despite the limitations of the present work due to its observational nature, the reported findings will augment Lp(a)-related research towards more focused investigation on the most vulnerable groups to provide more robust conclusions for clinical practice.

About 1 billion people worldwide have been detected with elevated Lp(a) levels, over the generally accepted – in the atherothrombotic range – cut-off point of 30 to 50 mg/dL or 75 to 125 nmol/L.[9] In our sample we found that about 8.5% (9.5% in women *vs* 7.5% in men) of a random, representative sample of apparently

healthy individuals had an Lp(a) ≥50 mg/dL. Current metrics suggest that Lp(a) affects cardiac health of 1 out of 3 individuals, worldwide.[9] Nevertheless, there remains controversy regarding the shape of Lp(a) risk curve and the extent to which Lp(a) confers CVD risk, independently of other lipid markers used in current risk equations. Our findings are in line with recent meta-analyses which confirm the existence of a positive association between Lp(a) and incident combined CVD, coronary heart disease, ischemic stroke or mortality.[18-21] The added value of the present work is related with the level of adjustment in the applied analyses. In particular, we highlighted that Lp(a) ≥50 mg/dL is associated with approximately 2 times higher risk to develop a cardiac episode within a decade, after taking into account participants' lipid profile in terms of Lp(a)-corrected LDL-C, HDL-C and triglycerides, instead of the commonly used total cholesterol. Most importantly, we further adjusted for the use of statins as well as for the liver and kidney function due to their directly aggravating effect on cardiac health.

As it is equivocally important to appropriately define the reference groups for comparisons, it is useful to emphasize that our findings are not entirely consistent with the methodology followed in other previous works. More specifically, the vast majority of recent cohorts examine the effect of Lp(a) on first CVD event on the basis of tertiles, quartiles or quintiles, mostly using the group of participants with the lowest Lp(a) values e.g. Lp(a) <10 mg/dL as the reference group.[11, 22-25] Using the "healthiest" participants as the reference group of analysis may be a matter of bias. Here, with a sample of apparently healthy individuals with generally low Lp(a) metrics, such a method did not reveal any significant outcomes. On the other hand, when the clinically recommended threshold of Lp(a) ≥50 mg/dL was used and examined against Lp(a) <50 mg/dL, a significantly increased CVD risk was observed.[1] Interestingly, this value was the optimal cut-off point i.e. with the best discriminative ability against 10-year first CVD-event in both sexes. As supported in the literature, the higher the Lp(a) level, the greater the CVD risk. The European Atherosclerosis Society proposed the value of <50 mg/dl as an optimal level to define the Lp(a) status.[1] This suggested threshold is in line with the findings presented here. Nevertheless, what is currently underscored in the literature, is the risk of patients with levels between 25 and 50 mg/dL.[9] To this issue, the 2016 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia suggested the value of 30 mg/dL, particularly in patients with intermediate CVD risk or patients with premature CVD history.[26] In the present work, the lower cut-off point of 30 mg/dL was examined but did not reveal any significant outcomes.

The role of sex on the association between Lp(a) and risk for incident CVD remains a matter of inconsistency. In particular, the Emerging Risk Factors Collaboration published a meta-analysis in 2009 combining 24 studies and 72,683 individuals to show that a 1 standard deviation (3.5-fold) higher Lp(a) was associated with a hazard ratio of 1.14 for CVD mortality, without any significant differences between sexes.[18] This meta-analysis further confirms the hypothesis that Lp(a) is a promising emerging risk factor in CVD epidemiology.[18] Nevertheless, it is only within the last 5 years that the sex-hypothesis has been considered more seriously with cohort studies providing sex-based analyses regarding the role of Lp(a) on CVD onset albeit with contradicting remarks. In this context, considering the recent literature, the magnitude of interaction between Lp(a) and sex on CVD risk seems to be moderate. Very recent highlights by *Cook et al.* with data from Women's Health Initiative and Women's Health study suggested that Lp(a) was independently associated with long-term CVD incidence, yet only in case of women with hypercholesterolemia.[11] This finding was replicated in the female sample of the JUPITER clinical trial while in case of men, Lp(a) retained its significant aggravating effect even at lower total-cholesterol levels.[11] Apart from these highlights corresponding to the American population, a very recent combined analysis of 2 large-scale cohort studies with a Danish population suggested that Lp(a) is independently associated with long-term CVD mortality in both sexes, with a marginally higher effect size in men.[22] Additional analyses with different endpoints i.e. non-CVD mortality and all-cause mortality, revealed a retention of the aforementioned association only in men.[22] The findings of our work are in line with the conclusions generated by the aforementioned prospective studies, revealing that conventional lipid markers may have a strong moderating effect on the Lp(a)-CVD association in women (Figure 1). In addition to this, we further highlighted that Lp(a) measurement in daily clinical practice may have an added value in the prediction of CVD onset and/or as a therapeutic target only in case of men, while for women other lipid markers may be more important (Table 3). This claim contradicts previous hypotheses where Lp(a) was put under the umbrella of emerging biomarkers for women's cardiac health.[2]

In addition to the aforementioned observations, we further revealed – through sensitivity analysis – that apart from the hypercholesterolemia diagnosis as a moderator or mediator of the Lp(a)-CVD association, already suggested and examined in the literature[11], the lipid markers with probably the most evident interacting effect on the examined relation were HDL-C, ApoA1 and triglycerides. Much as this sensitivity analysis was applied to the total sample – for statistical power reasons – and not separately for men and women, such observations may partially explain the aforementioned sex-related remarks; indeed, as mentioned before a significant interaction between sex, Lp(a) and these lipid markers on the CVD risk was observed. Conventional lipid markers and their subclasses have been investigated, even inadequately, towards a sex-specific orientation. In this context, HDL-C and triglycerides have been suggested as biomarkers with better predictive ability for women. For example, including HDL-C in the SCORE modestly improved women' risk stratification.[27] Furthermore, in the Women's Health Study, the inverse association between HDL-C and primary CVD incidence remained significant across all LDL-C and apoB levels.[28] As for triglycerides, a meta-analysis revealed a stronger association of fasting triglycerides with mortality attributed to cardiac episodes in women.[29] Hence, we could make the hypothesis that the observed interacting effect of these lipid markers on Lp(a)-CVD relation may be partially sex-oriented.

Adherence to Mediterranean diet was suggested to be an important lifestyle mediator that can influence the association between Lp(a) and 10-year CVD onset. It is suggested that moderate to high adherence to Mediterranean may have a protective effect on the cardiac health of participants with increased Lp(a) levels. A similar finding has been revealed using the sample of the present work yet in terms of Lp(a) as a continuous variable[16]; here, we decided to reexamine the interaction potential of MedDietScore on a more strictly defined Lp(a) status using the clinically recommended cut-off point. It is widely known that the cardioprotective effect of the Mediterranean diet could be largely explained through the beneficial effects of this pattern on classical as well as emerging risk factors.[30-31] Investigators from the PREDIMED clinical trial suggested that 1-year intervention with Mediterranean diet reduced triglycerides and shifted the lipoprotein particle profile to a less atherogenic pattern.[32] Hence, although the effect of Mediterranean diet on lipidemic profile has been examined with several clinically significant remarks, its association with Lp(a) remains quite a novel concept with inconclusive evidence. In the hitherto literature, the observed relations rank from weak to modest, yet further studies are demanded to understand the complexity of the role of diet on lipidemic profile and particularly in specific lipid markers.[33]

Strengths and Limitations

This study has several limitations. Only baseline measurements were taken into account; hence misclassifications of transitions misclassification of transitions cannot be precluded due to the extended interim periods between follow-up assessments. Additionally, much as sensitivity analyses were performed, the relatively small number of CVD events prevented us from conducting extensive subgroup analysis. Finally, since we examined whether Lp(a) improves risk prediction in a sample with a generally low CVD risk i.e. without established cardiac disease, the role of Lp(a) as a potentially useful clinical measure in patients with established CVD or those with a history of premature CVD could not be excluded.[33-36]

This study also has several strengths. First, we evaluated the sex-based effect of Lp(a) on 10-year CVD onset after an extensive adjustment of conventional and novel risk factors; to the best of our knowledge the evidence-based data regarding this issue are inadequate. Secondly, this in one of the very few prospective studies that provided metrics for discrimination and classification parameters. Lastly, this work revealed outcomes from extensive sensitivity analyses in terms of not only sex but also lipid markers; in this context, we revealed that the observed aggravating effect of Lp(a) on cardiac health may be alleviated by specific lipid markers, especially in women.

Conclusions

While ever increasing efforts have sought to elucidate Lp(a) as a therapeutic target or risk-prediction biomarker, in the context of primary or secondary CVD prevention clinical recommendations remain to be guided with appropriate evidence, mostly from a sex-centered standpoint. Our work aimed to address some knowledge gaps towards this orientation. Our findings suggest that Lp(a) levels above the clinically accepted threshold were associated with increased CVD risk, irrespectively of other conventional lipid markers; this observation was more evident in men, a fact that should be further explored. However, in the case of women, some lipid markers, as well as triglycerides levels, seemed to mediate the aforementioned association of Lp(a)

on CVD risk observed in men. Concomitantly, since the modifications in risk prediction ranked from minimal to no improvement in both sexes and especially in women, routine screening for Lp(a) may have little utility in primary prevention, limited to specific subgroups; however, further multicenter, and adequately powered studies are needed to confirm or refute the generalization of the presented findings. Finally, we provided additional evidence on the hypothesis that Mediterranean diet alleviates the aggravating effect of Lp(a) on cardiac health. This is another potential mechanism, that has rarely been explored, by which this dietary pattern could exert its cardioprotective properties.

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Table 1. Baseline sociodemographic, lifestyle, clinical and biochemical factors and 10-year cardiovascular disease event of apparently healthy participants according to Lp(a) status (*n*=1,890).

	Lp(a) status, mg/dL		
	<50 (normal)	**≥50 (high)**	*p-value*
Baseline factors	*n=1,725*	*n=165*	
Age, years	44 (13)	45 (13)	*0.11*
Men, %	50	46	*0.20*
Years of school	12 (4)	11 (3)	*0.09*
Body mass index, kg/m^2	26.2 (4.5)	26.0 (4.1)	*0.46*
Current smoking, %	43	42	*0.66*
MedDietScore <27, %	59	50	*0.02*
Physical activity, no, %	61	58	*0.65*
History of hypertension, %	30	29	*0.93*
Lp(a)-corrected total cholesterol, mg/dL	192 (41)	203 (42)	*<0.001*
Lp(a)-corrected LDL-C, mg/dL	116 (43)	114 (36)	*0.001*
ApoB, mg/dL	111 (41)	124 (21)	*<0.001*
HDL-C, mg/dL	46 (20)	52 (22)	*0.10*
ApoA1, mg/dL	152 (31)	157 (31)	*0.21*
Triglycerides, mg/dL	99 (70)	104 (77)	*0.77*
Lipid-lowering drugs, %	6	8	*0.04*
History of diabetes mellitus, %	6	5	*0.39*
C-Reactive Protein, mg/L	1.05 (2.11)	1.95 (1.10)	*0.04*
Alanine transaminase, U/L	17.00 (10.00)	17.00 (9.00)	*0.74*
Aspartate transaminase, U/L	22.00 (9.00)	21.00 (9.00)	*0.18*
Creatinine clearance, mL/min/1.73m^2	83 (32)	87 (38)	*0.001*
Family history of CVD, %	28	27	*0.71*
10-year follow-up			
First combined cardiovascular disease event, %	14	24	*0.05*

Data are presented as mean (standard deviation) for normally distributed variables or median (Interquartile Range) for not normally distributed variables. P-values were obtained using Student's t-test for independent samples for the normally distributed variables (age, body mass index), Mann Whitney Test for the rest quantitate variables (years of school, triglycerides, HDL-C, ApoA1, Lp(a)-corrected LDL-C, ApoB100, Lp(a)-corrected total cholesterol, C-Reactive Protein, Creatinine clearance, Aspartame transaminase, Alanine transaminase and chi-squared test for categorical variables. Total cholesterol and LDL-C were corrected for the Lp(a) contribution by subtracting 30% of total Lp(a) mass. ***Abbreviations:*** Apolipoprotein A1 (ApoA1); Apolipoprotein B100 (ApoB); high density lipoprotein cholesterol (HDL-C); Lipoprotein (a) (Lp(a)); low density lipoprotein cholesterol (LDL-C)

Table 2. Cox-regression models to evaluate the association of abnormal Lp(a) levels (cut-off point of 50 mg/dL) with 10-year cardiovascular disease risk (*n*=1,890).

	Model 1 HR (95%CI)	**Model 2** HR (95%CI)	**Model 3** HR (95%CI)	**Model 4** HR (95%CI)	**Model 5** HR (95%CI)
Lp(a), ≥50 mg/dL *vs* <50 mg/dL	2.65 (2.01, 4.02)***	2.43 (1.93, 3.94)**	2.34 (1.80, 3.80)**	2.21 (1.16, 4.21)*	2.18 (1.11, 4.28)*
Age, per 1 year	-	1.06 (1.05, 1.07)	1.06 (1.05, 1.07)	1.04 (1.03, 1.06)	1.04 (1.02, 1.07)
Male *vs* female sex	-	1.75 (1.38, 2.22)	1.65 (1.29, 2.12)	1.51 (1.04, 2.21)	1.63 (0.98, 2.70)
Body mass index, per 1 kg/m^2	-	-	1.03 (1.01, 1.06)	1.04 (1.01, 1.08)	1.01 (0.96, 1.06)
Current smoking, yes *vs* no	-	-	1.35 (1.04, 1.76)	1.05 (0.72, 1.53)	1.09 (0.69, 1.72)
Diabetes mellitus, yes *vs* no	-	-	1.52 (1.10, 2.09)	1.77 (1.11, 2.80)	1.77 (0.95, 3.34)
Hypertension, yes *vs* no	-	-	1.05 (0.81, 1.35)	1.04 (0.72, 1.48)	1.06 (0.81, 1.97)
MedDietScore, <27 *vs* ≥27	-	-	0.89 (0.46, 1.34)	0.92 (0.47, 1.36)	0.92 (0.47, 1.36)
Lp(a)-corrected LDL-C, per 1 mg/dL	-	-	-	1.00 (0.99, 1.01)	1.01 (1.00, 1.02)
HDL-C, per 1 mg/dL	-	-	-	0.98 (0.97, 1.00)	0.98 (0.97, 1.00)
Triglycerides, per 1 mg/dL	-	-	-	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)
Use of statins, yes *vs* no	-	-	-	2.20 (1.45, 3.34)	2.53 (1.44, 4.00)
Family history of cardiovascular disease, yes *vs* no	-	-	-	-	1.00 (0.61, 1.63)
CRP, per 1 mg/L	-	-	-	-	1.09 (1.02, 1.18)
ALT, per 1 U/L	-	-	-	-	1.00 (0.97, 1.04)
AST, per 1 U/L	-	-	-	-	0.98 (0.94, 1.01)
$C_{(CR)}$, per 1 mL/min/1.73m^2	-	-	-	-	0.99 (0.98, 1.01)

****p-value<0.001*; ***p-value<0.01*; **p-value<0.05*

LDL-C was corrected for the Lp(a) contribution by subtracting 30% of total Lp(a) mass. ***Abbreviations:*** Alanine transaminase (ALT); Aspartate transaminase (AST); 95% Confidence Interval (95%CI); C-Reactive Protein (CRP); Creatinine clearance ($C_{(CR)}$); Hazard Ratio (HR); High density lipoprotein cholesterol (HDL-C); Lipoprotein(a) (Lp(a)); Low density lipoprotein cholesterol (LDL-C)

Table 3. Cox-regression models to evaluate the association of abnormal Lp(a) levels (cut-off point of 30mg/dL) with 10-year cardiovascular disease risk (*n*=1,890).

	Model 1 HR (95%CI)	**Model 2** HR (95%CI)	**Model 3** HR (95%CI)	**Model 4** HR (95%CI)	**Model 5** HR (95%CI)
Lp(a), ≥30 mg/dL *vs* <30 mg/dL	1.31 (0.81, 2.12)	1.22 (0.90, 1.66)	1.20 (0.85, 1.68)	1.18 (0.82, 1.60)	1.18 (0.82, 1.60)
Age, per 1 year	-	1.06 (1.04, 1.08)	1.06 (1.05, 1.07)	1.04 (1.03, 1.06)	1.04 (1.02, 1.07)
Male *vs* female sex	-	1.76 (1.39, 2.20)	1.64 (1.27, 2.13)	1.51 (1.04, 2.21)	1.63 (0.98, 2.70)
Body mass index, per 1 kg/m^2	-	-	1.02 (1.01, 1.06)	1.05 (1.01, 1.09)	1.01 (0.95, 1.06)
Current smoking, yes *vs* no	-	-	1.37 (1.05, 1.78)	1.06 (0.72, 1.54)	1.09 (0.69, 1.72)
Diabetes mellitus, yes *vs* no	-	-	1.52 (1.10, 2.09)	1.77 (1.11, 2.80)	1.77 (0.95, 3.34)
Hypertension, yes *vs* no	-	-	1.05 (0.81, 1.35)	1.04 (0.72, 1.48)	1.06 (0.81, 1.97)
MedDietScore, <27 *vs* ≥27	-	-	0.87 (0.44, 1.33)	0.90 (0.46, 1.37)	0.93 (0.46, 1.36)
Lp(a)-corrected LDL-C, per 1 mg/dL	-	-	-	1.00 (0.99, 1.01)	1.01 (1.00, 1.02)
HDL-C, per 1 mg/dL	-	-	-	0.98 (0.97, 1.00)	0.98 (0.97, 1.00)
Triglycerides, per 1mg/dL	-	-	-	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)
Use of statins, yes *vs* no	-	-	-	2.20 (1.45, 3.34)	2.53 (1.44, 4.00)
Family history of cardiovascular disease, yes *vs* no	-	-	-	-	1.01 (0.62, 1.61)
CRP, per 1 mg/L	-	-	-	-	1.10 (1.01, 1.17)
ALT, per 1 U/L	-	-	-	-	1.00 (0.97, 1.04)
AST, per 1 U/L	-	-	-	-	0.98 (0.94, 1.01)
$C_{(CR)}$, per 1 mL/min/1.73m^2	-	-	-	-	0.98 (0.97, 1.01)

****p <0.001*; ***p <0.01*; **p <0.05*

LDL-C was corrected for the Lp(a) contribution by subtracting 30% of total Lp(a) mass. ***Abbreviations:*** Alanine transaminase (ALT); Aspartate transaminase (AST); 95% Confidence Interval (95%CI); C-reactive protein (CRP); Creatinine clearance ($C_{(CR)}$); Hazard Ratio (HR); High density lipoprotein cholesterol (HDL-C); Lipoprotein(a) (Lp(a)); Low density lipoprotein cholesterol (LDL-C)

Table 4. Discrimination-ability parameters of multivariate models adjusted for conventional lipid markers or Lipoprotein (a) or the combination of them over the 10-year first fatal/non-fatal cardiovascular disease event (*n*=1,890).

Models	Model adjustment description	C-index (95%CI)	Correct classification rate, % (total)	Correct classification rate, % (cases)
Model 1	Standard model* adjusted for **conventional lipid markers**[l]		**Men**	
		0.772 (0.713, 0.831)	83.6	33.3
			Women	
		0.831 (0.777, 0.886)	89.6	19.6
Model 2	Standard model* adjusted for **Lipoprotein (a)**		**Men**	
		0.769 (0.709, 0.828)	81.9	24.8
			Women	
		0.820 (0.772, 0.880)	87.9	8.5
Model 3	Standard model* adjusted for **all lipid markers**		**Men**	
		0.784 (0.725, 0.839)	96.5	32.2
			Women	
		0.829 (0.774, 0.883)	88.7	15.7

*Standard model was adjusted for age, body mass index, current smoking, MedDietScore, hypertension, diabetes mellitus, family history of cardiovascular disease.
[l]Conventional lipid markers examined were lipoprotein (a) corrected low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglycerides.
C-index and the corresponding confidence interval was evaluated through the area under the curve obtained from the Receiver operating Characteristics (ROC) analysis. ROC analysis was performed using the probabilities for 10-year first fatal/non-fatal cardiovascular disease event, corresponding to each study participant, separately for men and women, calculated from Cox regression analysis using the multivariate models described. Correct classification rate was obtained from the Cox regression analysis performed using the described models, separately for men and women.

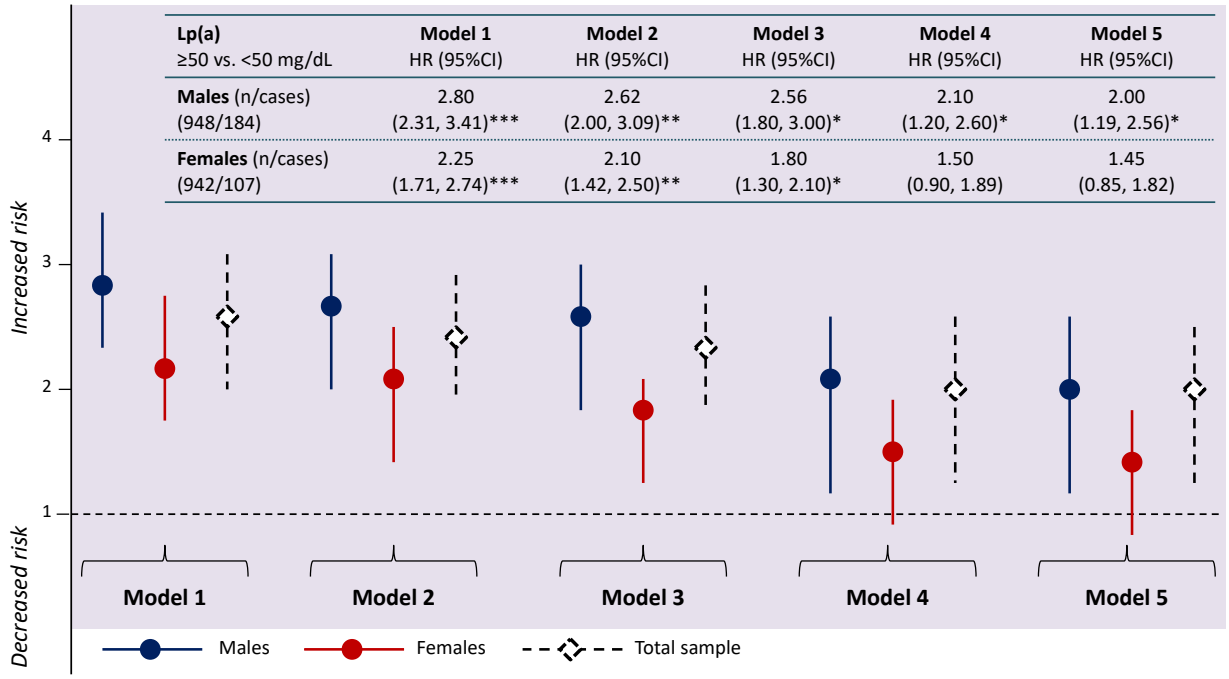
Table 5. Sensitivity analyses to evaluate the association of Lp(a) status (cut-off point of 50 mg/dL) with 10-year cardiovascular disease risk in specific subgroups (*n*=1,890).

	Lp(a) status	Hazard ratio	95% Confidence Interval
Lp(a)-corrected total cholesterol (n/cases)			
<200 mg/dL (1,057/117)	≥50 *vs* <50 mg/dL	1.42	0.62, 3.29
≥200 mg/dL (833/174)		**1.91**	**1.10, 4.00**
p for interaction=0.005			
Lp(a)-corrected LDL-C (n/cases)			
LDL-C <100 mg/dL (624/30)	≥50 *vs* <50 mg/dL	2.05	0.81, 5.22
LDL-C ≥100 mg/dL (1,266/261)		1.72	0.92, 3.21
p for interaction=0.87			
HDL-C (n/cases)			
HDL-C ≥40 mg/dL in men and ≥50 mg/dL in women (1,076/128)	≥50 *vs* <50 mg/dL	1.24	0.55, 2.76
HDL-C <40 mg/dL in men and <50 mg/dL in women (814/163)		**1.89**	**1.01, 3.55**
p for interaction=0.01			
Triglycerides (n/cases)			
Triglycerides <150 mg/dL (1,451/113)	≥50 *vs* <50 mg/dL	1.39	0.75, 2.57
Triglycerides ≥150 mg/dL (439/178)		**2.15**	**1.37, 5.26**
p for interaction=0.005			
ApoB (n/cases)			
ApoB <100 mg/dL (805/74)	≥50 *vs* <50 mg/dL	1.82	0.47, 4.01
ApoB ≥100 mg/dL (1,085/217)		1.75	0.93, 2.98
p for interaction=0.12			
ApoA1 (n/cases)			
ApoA1 ≥120 mg/dL for men and ≥140 mg/dL for women (1,621/246)	≥50 *vs* <50 mg/dL	1.28	0.79, 5.31
ApoA1 <120 mg/dL for men and <140 mg/dL for women (269/45)		**1.86**	**1.12, 3.10**
p for interaction=0.002			
MedDietScore (n/cases)			

MedDietScore <27 (892/259)		**1.90**	**1.08, 3.33**
	≥50 *vs* <50 mg/dL		
MedDietScore ≥27 (707/32)		1.11	0.55, 5.12
p for interaction=0.001			

LDL-C was corrected for the Lp(a) contribution by subtracting 30% of total Lp(a) mass. Cut-off values of the lipids-oriented strata were set according to the "2016 ESC/EAS Guidelines for the Management of Dyslipidaemias". All models were adjusted for age, sex, current smoking, family history of cardiovascular disease, C-reactive protein, alanine transaminase, aspartate transaminase and creatinine clearance. **Bold** indicates estimates that are significantly different from the reference group at $p<0.05$.
Abbreviations: Apolipoprotein A1 (ApoA1); Apolipoprotein B100 (ApoB); High density lipoprotein cholesterol (HDL-C); Lipoprotein(a) (Lp(a)); Low density lipoprotein cholesterol (LDL-C)

Figure 1 Nested Cox-regression analysis to evaluate the association between Lipoprotein (a) status (cut-off point of 50 mg/dL) and the 10-year cardiovascular disease event, separately for men and women.



HRs (dots) and their corresponding 95%CIs (vertical lines) for Lipoprotein (a) ≥50 mg/dL *vs* <50 mg/dL were obtained through Cox regression analysis. *Model 1:* crude model; *Model 2:* age; *Model 3:* Model 2 plus body mass index, current smoking, hypertension, diabetes mellitus, MedDietScore; *Model 4:* Model 3 plus lipoprotein(a)-corrected low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides, use of statins; *Model 5:* Model 4 plus C-reactive protein, alanine transaminase, aspartate transaminase, creatinine clearance, family history of cardiovascular disease. ***Abbreviations:*** Hazard ratio (HR), 95% Confidence Interval (95%CI). ****p <0.001; **p <0.01; *p <0.05*