Aggregation of lipoprotein(a) to apolipoprotein A-I underlying HDL dysfunction as a major coronary risk factor

HDL disfonksiyonunun temelindeki lipoprotein(a)'nın apolipoprotein A-I'e agregasyonu majör bir koroner risk faktörüdür

Altan Onat, Günay Can[1], Sani Murat[2], Gökhan Çiçek[3], Ender Örnek[2], Hüsniye Yüksel[1]

Department of Cardiology, Turkish Society of Cardiology, Cerrahpaşa Faculty of Medicine, İstanbul-*Turkey*
[1]Department of Public Health, Cerrahpaşa Faculty of Medicine, İstanbul University, İstanbul- *Turkey*
[2]Clinic of Cardiology, Etlik İhtisas Education Hospital, Ankara-*Turkey*
[3]Clinic of Cardiology, Siyami Ersek Center for Cardiovascular Surgery, İstanbul-*Turkey*

ABSTRACT

Objective: Dysfunction of high-density lipoprotein (HDL) may contribute to coronary heart disease (CHD) risk. We determined whether aggregation to lipoprotein (Lp)(a) of apolipoprotein (apo) A-I underlies HDL dysfunction conferring incident CHD risk.
Methods: A representative sample of 1509 middle-aged Turkish adults was studied at 4.9-years' follow-up yielding 198 incident CHD cases. Statistical analysis was performed using multiple linear regression and Cox proportional regression analyses.
Results: In women, not age or apoA-I, rather complement C3, apoE levels and statin use were linearly related to log-Lp(a). Individuals in the low Lp(a) tertile (<6.4 mg/dL) displayed high mean triglyceride and apoE values, and geometric mean Lp(a) values increased moderately in subjects having low and mid tertiles of apoE or triglycerides, only to be lower in the high tertiles ($p \leq 0.002$). These two findings indicated the unexpected fall in Lp(a) under circumstances of high apo E (>4.5 mg/dL) and/or triglycerides (>2.0 mmol/L). Levels actually represent aggregation of Lp(a) to apoA-I in an immune complex, rendering apoA-I atherogenic. Lp(a) did not, but apoA-I did significantly predict incident CHD (HR 1.21 [95%CI 1.07; 1.37]) in Cox regression analyses after adjustment for conventional risk factors and statin use. This adverse action of apoA-I was independent of prevalent metabolic syndrome (MetS), existed in individuals in whom ATPIII-defined MetS was not identified, and was similar in magnitude to that of conventional risk factors.
Conclusion: Beyond being atherogenic, Lp(a) may aggregate in a pro-inflammatory milieu to apoA-I, rendering apoA-I atherogenic. This process is independent of ATPIII-defined MetS and exhibits risk magnitude similar to that of conventional risk factors.
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Key words: Apolipoprotein A-I, apolipoprotein E, coronary heart disease, HDL dysfunction, lipoprotein(a), metabolic syndrome, regression analysis

ÖZET

Amaç: Yüksek yoğunluklu lipoprotein (HDL) disfonksiyonu koroner kalp hastalığına (KKH) katkıda bulunabilir. Yeni gelişen KKH riskine yük bindiren HDL disfonksiyonunun altında lipoprotein [Lp](a)'nın apolipoprotein (apo) A-I'e agregasyonunun yatabileceği keyfiyetini araştırdık.
Yöntemler: Orta yaşlı Türk yetişkinlerini temsil eden 1509 kişi, 198 yeni KKH'nın geliştiği 4.9 yıllık takipte incelendi. İstatistiksel analiz çoklu regresyon ve Cox orantısal regresyon analizleri ile yapıldı.
Bulgular: Kadınlarda, yaş veya apoA-I değil, kompleman C3 ile apoE düzeyleri ve statin kullanımının log-Lp(a) ile doğrusal ilişkisi vardı. Lp(a) alt üçte bir dilimindeki (<6.4 mg/dL) bireyler yüksek ortalama trigliserid ve apo E değerleri sergiledi. Geometrik ortalama Lp(a) değerleri, apoE veya trigliserid düşük ya da orta üçte bir dilimlerindeki kişilerde ılımlı ölçüde yüksekken, yüksek üçte bir dilimde daha düşüktü ($p \leq 0.002$). Bu iki bulgu, yüksek apoE (>4.5 mg/dL) ve/veya trigliserid'li (>2.0 mmol/L) koşullarda Lp(a)'nın beklenmeyen düşüşünü işaret etmekteydi. Düzeyler aslında Lp(a)'nın apoA-I ile bileşmesinden oluşan ve apoA-I'i aterojen kılan bir immün kompleksi simgeliyordu. Cox regresyonu analizinde, geleneksel risk faktörleri ve statin kullanımı için ayarlandıktan sonra, yeni gelişen KKH'yı, Lp(a) değil, apoA-I anlamlı olarak öngördü (HR 1,21 [%95 CI 1,07; 1.37]). ApoA-I'in bu olumsuz etkisi metabolik sendrom (MetS) varlığından bağımsız olup ATPIII-tanımlı MetS belirlenmemiş bireylerde de gözlemlendi ve etki konvansiyonel risk faktörlerinin boyutuna benzerlik gösterdi.

Address for Correspondence/Yazışma Adresi: Dr. Altan Onat, Nisbetiye Cad. 59/24, 34335 Etiler, İstanbul-*Türkiye*
Phone: +90 212 351 62 17 E-mail: alt_onat@yahoo.com.tr
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Sonuç: Lp(a), aterojen olma ötesinde, artmış yangı ortamında apoA-I'e onu aterojen kılacak biçimde agregasyon yapabilir. Bu süreç, ATPIII-tanımlı MetS'den bağımsız olup sergilediği riskin boyutu konvansiyonel risk faktörlerininkine benzerdir.
(Anadolu Kardiyol Derg 2013; 13: 543-51)
Anahtar kelimeler: Apolipoprotein A-I, apolipoprotein E, koroner kalp hastalığı, HDL disfonksiyonu, lipoprotein(a), metabolik sendrom, regresyon analizi

Introduction

We had documented in a general population that high apolipoprotein (apo) A-I concentrations, presumably rendered to lack anti-inflammatory and atheroprotective activities, significantly predicted type-2 diabetes independent of waist circumference and other relevant confounders (1). We have also reported epidemiologic evidence of the presence of dysfunction of high-density lipoprotein (HDL) particles in the Turkish Adult Risk Factor (TARF) study cohort (2) and reviewed evidence that such impaired function was a major determinant of cardiometabolic risk also in some other populations or population subsets prone to impaired glucose tolerance (3). Dysfunction of apoA-I leading to type-2 diabetes was observed to be independent of the apoE genotype (4).

Excess lipoprotein (Lp)(a) is known from animal (5) and epidemiologic (6) studies to promote thrombosis, inflammation and coronary artery disease. A notable previous finding that the low compared to mid-tertile of Lp(a) concentrations were independently associated with higher fasting triglyceride levels and likelihood of metabolic syndrome (MetS) (7), prompted us to hypothesize the following: an inverse association of Lp(a) may appear in pro-inflammatory state/oxidative stress, whereby low Lp(a) levels might actually represent inassayability of damaged Lp(a) in an autoimmune process that involves serum apoA-I and results from pro-inflammatory state/oxidative stress. Indeed, apoA-I has recently been reported to be combined during oxidation to low-density lipoprotein (LDL) (apoAI-LDL), high levels of which could mark in a cross-sectional study coronary artery disease more accurately than C-reactive protein (CRP) (8).

As an LDL-like particle consisting of an apoB100 molecule linked to a glycoprotein, apolipoprotein(a), Lp(a) has been recognized to be in very weak correlation with other lipid and non-lipid parameters (6, 9, 10). This particle's function remains largely uncertain; Lp(a) binds proinflammatory-oxidized phospholipids (11) and is a preferential carrier of oxidized phospholipids (ox-PL) in human plasma. Lp(a) also contains lipoprotein-associated phospholipase A_2 (Lp-PLA_2) which may cleave oxidized fatty acids to yield short-chain fatty acids and lysolecithin (12).

A recent consensus statement (of the European Atherosclerosis Society) (12) concurred with the meta-analysis by the Emerging Risk Factors Collaboration (ERFC) (9) and concluded that the association between circulating Lp(a) and cardiovascular disease risk is independently continuous, without a threshold. This conclusion may not be applicable to population subsets prone to impaired glucose tolerance (IGT) (see Discussion). Indeed, in such a cohort, apoA-II influenced apoE-linked cardiovascular disease in Dutch women with high levels of HDL-cholesterol and C-reactive protein (13).

We addressed to clarify the above-stated hypothesis in the TARF participants with the primary objective of: a) jointly analyzing prospectively apoA-I and Lp(a) levels along with conventional risk factors in predicting incident CHD, possibly identifying the presence of such associations after stratification to gender and MetS: and the secondary objective of: b) scrutinizing the cross-sectional relationship of (low) Lp(a) levels to biomarkers of enhanced systemic low-grade inflammation (CRP, C3, apoE), apoA-I and fasting triglycerides. Such an investigation might elucidate novel pathogenetic mechanisms in cardiometabolic risk carrying implications for prevention.

Methods

Population sample

The *Turkish Adult Risk Factor Study* is a prospective survey on the prevalence of cardiac disease and risk factors in adults in Turkey carried out periodically almost biennially since 1990 in 59 communities scattered throughout all geographical regions of the country (14). It involves a random sample of the Turkish adult population, representatively stratified for sex, age, geographical regions and for rural-urban distribution (14). Combined measurements of serum apoA-I and Lp(a) were first performed at the follow-up visit in 2002. The survey conformed to the principles embodied in the Declaration of Helsinki and was approved by the İstanbul University Ethics Committee. Written informed consent was obtained from all participants. Data were obtained by history of the past years via a questionnaire, physical examination of the cardiovascular system, sampling of blood and recording of a resting electrocardiogram.

Measurements of risk variables

Blood pressure (BP) was measured in the sitting position on the right arm, and the mean of two readings at least 3 min apart was recorded. Waist circumference was measured with a tape, the subject standing and wearing only underwear, at the level midway between the lower rib margin and the iliac crest.

Blood samples were collected, spun at 1000*g* and were stored in deep-freeze at -75°C until analyzed in a central laboratory. Serum concentrations of Lp(a), apoE, apoA-I apoB and complement C3c were measured after an overnight fast with kits and by nephelometry (Behring Diagnostics, Marburg, Germany, or Westwood, MA). Serum concentrations of total cholesterol, fasting triglycerides, glucose, and HDL-cholesterol (HDL-C plus 2nd generation, direct quantification) were determined using enzymatic kits from Roche Diagnostics with a Hitachi 902 autoanalyzer. LDL-cholesterol values were computed with the Friedewald formula. Serum gamma glutamyltransfer-

ase (GGT) activity was assayed by the kinetic method using Glucana as substrate (Thermo Trace, Noble Park, Victoria, Australia). Fibrinogen levels were assayed in plasma by the modified Clauss method using Behring Fibrintimer II coagulometer and Multifibren U kit.

Definitions and outcomes

Individuals with metabolic syndrome were identified when 3 out of the 5 criteria of the National Cholesterol Education Program (ATP III) were met, modified for prediabetes fasting glucose 5.56-6.95 mmol/L (15) and further for male abdominal obesity using as cut-point ≥95 cm (16).

Information on the mode of death was obtained from first-degree relatives and/or health personnel of local health office. Cause of death was assigned with the consideration of pre-existing clinical and laboratory findings elicited during biennial surveys. CHD death comprised death from heart failure of coronary origin and fatal coronary event. Nonfatal *CHD* was identified by presence of angina pectoris, a history of myocardial infarction with or without accompanying Minnesota codes of the electrocardiogram (ECG) (17) or a history of myocardial revascularization. Typical angina and, in women, age >45 years were prerequisite for a diagnosis when angina was isolated. ECG changes of "ischemic type" of greater than minor degree (Codes 1.1-2, 4.1-2, 5.1-2, 7.1) were considered as myocardial infarct sequelae or myocardial ischemia, respectively.

Statistical analysis

Statistical analyses were performed using SPSS-10 for Windows (SPSS Inc., Chicago, IL). Tertiles were formed by cut-points of 6.4 and 17.3 mg/dL of Lp(a), by 3.44 and 4.5 mg/dL of apoE and by 111 and 180 mg/dL of fasting triglyceride values. Descriptive parameters were shown as mean (± standard deviation) or adjusted estimated mean (± standard error) or in percentages. Due to the skewed distribution, log-transformed (geometric) values were used for Lp(a), GGT and apoE. Two-sided *t*-tests and Pearson's Chi-square tests were used to analyze the differences between means and proportions between groups. Multiple linear regression analyses were performed with continuous parameters, expressed in terms of an increment of 1 SD. After exclusion of the cohort with prevalent CHD at baseline examination, Cox proportional hazards regression models were used for incident CHD at a mean follow-up of 4.9 years. Risk estimates (hazard ratio, HR) and 95% confidence intervals (CIs) were obtained in models that adjusted for confounders, again expressed in terms of 1 SD increment. Proportionality was upheld between the independent variables and follow-up time. p<0.05 on the 2-tailed test was considered statistically significant.

Results

Baseline measurements of serum Lp(a) and apoA-I were available in 1509 adults; excluded were 79 cases with prevalent CHD and 100 subjects with no follow-up. The latter was 4.9±1.8 years during which CHD newly developed in 198 cases. Baseline characteristics presented in Table 1 indicate overall relatively wide waist girths, high serum triglyceride, normal total cholesterol and apoA-I, low HDL-cholesterol, slightly low geometric mean Lp(a) concentrations.

Lp(a) concentrations and associations with pro-inflammatory markers

Median Lp(a) value at baseline was 10.1 (IQ range 4.46 and 21.9) mg/dL. With the aim of testing log-linearity, observed versus expected Lp(a) values were plotted (Fig. 1) which depicted substantial amount of lacking values >40 mg/dL, and accumulated values <15 mg/dL in excess of expected ones, a constellation in agreement with the current hypothesis of aggregation of part of Lp(a).

Complement C3 and GGT (measured in 978 and 1258 subjects) showed similar GGT (28.6 vs 28.7 g/L, p=0.94) but higher C3 values (1.38 vs 1.31 g/L, p=0.007) in the high Lp(a) category (≥30 mg/dL, constituting 17.5% of subjects).

A significant (p<0.001) measure of agreement, kappa, was observed in men +0.22 and in women +0.21 (total of 953 adults) between the percentage distribution of fasting apoE tertiles and that of triglyceride tertiles, by gender (Fig. 2).

Table 1. Baseline characteristics of the sample population by gender (n= 1509)

Variables	Men (n= 698)			Women (n=811)		p
	n	mean	SD	mean	SD	
Age, years	1509	53.4	11.4	53	11.5	ns
Waist circumference, cm	1501	94.4	10.8	92.7	12.7	**
Systolic BP, mmHg	1486	126. 7	20.8	132.2	24.8	**
Diastolic BP, mmHg	1486	81.5	12.4	82.6	13.6	ns
Fasting glucose, mmol/L	1082	5.48	1.94	5.49	1.9	ns
Total cholesterol, mmol/L	1507	4.80	0.96	5.09	1.02	**
Fast. triglycerides¶, mmol/L	1003	1.65	1.70	1.41	1.60	**
HDL-cholesterol, mmol/L	1507	0.98	0.28	1.20	0.32	**
Apolipoprotein A-I, g/L	1509	1.28	0.33	1.41	0.30	**
Apolipoprotein B, g/L	1500	1.10	0.34	1.09	0.34	ns
Lipoprotein(a) ¶, µmol/L	1509	0.31	0.10	0.42	0.10	**
Apolipoprotein E, mg/dL	864	4.27	1.9	4.24	1.60	ns
Complement C3, g/L	885	1.30	0.27	1.35	0.28	ns
γ-glutamyltransferase, U/L¶	1145	26.5	1.87	19.0	1.82	**
Current/former smoking, %	1470	51	22	14.5	3.3	**
Diabetes type-2, n, %	1468	32	4.7	38	4.8	ns
Use of statins†, n, %	1509	40	5.7	75	9.2	*

¶Geometric means p*<0.05, **<0.01 ns= not significant
†Includes subjects with prevalent coronary heart disease
Data are presented are mean/SD and number percentage
t-test for independent samples and Chi-square test
BP - blood pressure, Fast. - fasting, HDL - high-density lipoprotein

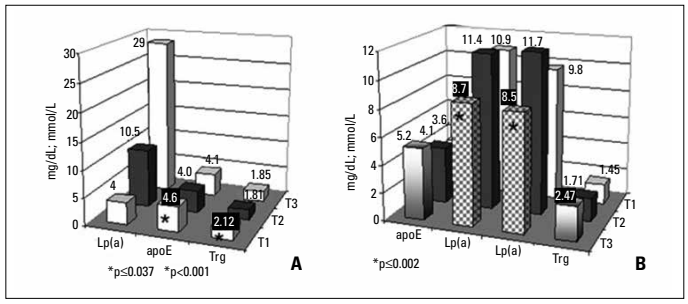

Figure 1. A) Comparative distribution of mean serum apoE levels and fasting triglycerides (in 1003 adults) by decreasing lipoprotein (a) tertiles. Significantly increased levels of circulating apo E as well as of triglycerides (indicated with asterix) are noted in participants with the lowest Lp (a) tertiles. B) Distribution of mean serum levels of Lp (a) in 1003 adults with increasing fasting triglyceride and with increasing apoE tertiles. Noteworthy is -following an increase in the mid-tertile - the highly significant marked fall in Lp (a) levels in the highest tertile of each of the two parameters

apo - apolipoprotein, Lp - lipoprotein

Table 2. Linear regression analysis for covariates of serum lipoprotein(a)¶ (n=477)

	Men & women			Women (n=268)		
	β-coeff.	SE	p	β-coeff.	SE	p
Sex, female	1.31	1.11	0.011			
Age, 11 years	1.02	1.007	0.97	1.02	1.007	0.79
Complement C3, 0.27 g/L	1.08	1.05	0.13	1.16	1.07	0.027
Apolipoprotein E, 0.015 g/L ¶	0.94	1.028	0.015	0.80	1.007	0.003
Apolipoprotein A-I, 0.35 g/L	1.04	1.08	0.55	1.03	1.08	0.73
Lipid-lowering drugs	1.71	1.20	0.004	2.11	1.25	0.001
Explained variance (r^2)	0.05 (p=0.001)			0.07 (p=0.001)		

The model was not significant in 209 men: p=0.81

¶ log-transformed values

Units following the independent variables denote 1 standard deviation.

Significant values are highlighted in boldface

In 1504 people who had measurements of both Lp(a) and triglycerides, triglycerides tertiles were inversely related to Lp(a) tertiles, so that individuals with low Lp (a) tertile (<6.4 mg/dL) were associated with high mean triglyceride (p<0.001 from both other two tertiles), as well as high mean apoE values (p<0.008 and 0.037 from those in the mid and high Lp(a) tertiles), measured in 864 subjects (Fig. 1A).

Panel B in Figure 1 shows that Lp(a) geometric mean values increased moderately in subjects comprised in the low and mid tertiles of apoE and triglycerides, only to drop in the high tertiles (by 24% and 27%, p≤0.002). These two findings indicate, collectively, that an unexpected "fall" was associated in Lp(a) under circumstances with high levels of apoE (>4.5 mg/dL) and/or triglycerides (>2.0 mmol/L).

A multiple linear regression analysis was performed for covariates of Lp(a) in 477 subjects (Table 2) in whom sex, age, use

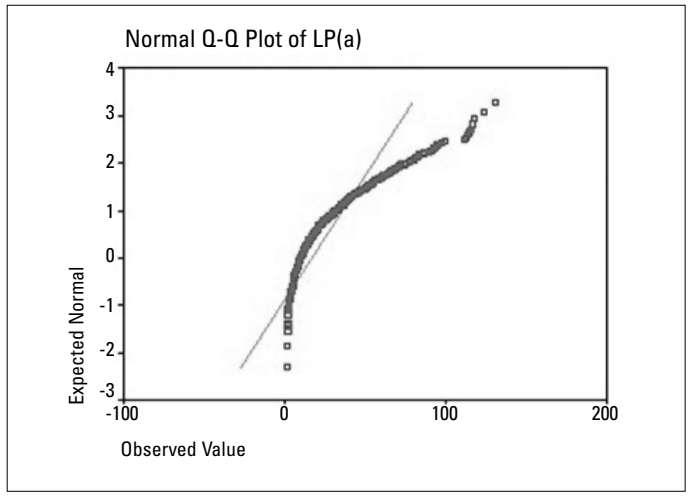

Figure 2. Observed values of Lp (a) plotted against logarithmic expected values (straight line). Values >40 mg/dL in deficitary numbers, and overly accumulated values <15 mg/dL are noteworthy

Lp - lipoprotein

of lipid-lowering drugs, complement C3, apo E and apo A-I were selected as independent variables comprised in our hypothesis; the model proved highly significant in the total sample and in women, not in men. Female sex, use of lipid-lowering drugs and complement C3 were positively associated, while 1 SD increment in apoE was associated with 20% lower Lp(a) levels.

When similarly classified Lp(a) concentrations were compared with variables at the final examination (mean 3 years later), subjects in the high category (≥30 mg/dL) compared with the remainder of the sample had higher apoB, total and LDL-cholesterol and fibrinogen values (p in each ≤0.002), but also by 0.06 mmol/L higher (=dysfunctional) HDL-cholesterol (p=0.005), apoA-I (by 0.03 g/L, p=0.076) and lower triglyceride (by 0.14 mmol/L; p=0.017) values.

Cox analysis for incident CHD

Table 3 shows results of Cox regression analysis for incident CHD in the total sample, stratified further by gender and presence of MetS. Hazard ratios of conventional risk factors (age, systolic BP, nonHDL-cholesterol and diabetes) except for smoking are in agreement with published meta-analyses on Western populations when adjusted for inflammatory biomarkers. Current smoking was not significantly associated. HDL-cholesterol displayed limited protection against CHD while apoA-I conferred independent CHD risk in magnitude close to nonHDL-cholesterol or systolic BP. Statin use contributed additive CHD risk in women with MetS. Lp(a) was not significantly involved (though tended to confer marginal risk in men).

People with compared to without MetS exhibited attenuation of HRs relative to systolic BP, apoA-I and diabetes, in males also to nonHDL-cholesterol. Women tended to low HRs with respect to BP and apoA-I. MetS significantly conferred CHD risk (HR 1.82) additively to the conventional risk factors, apoA-I and diabetes (Table 4).

Table 3. Cox regression analysis for prediction of incident CHD by presence or absence of MetS

Variables	Total			Men			Women		
	HR	95% CI		HR	95% CI		HR	95% CI	
Total	198/1330†			89/607†			109/723†		
Apolipoprotein A-I, 0.35 g/L	1.21	1.07;	1.37	1.21	1.07;	1.37	1.12	0.90;	1.42
HDL-cholesterol, 0.31 mmol/L	0.82	0.71;	0.96	0.80	0.64;	1.01	0.87	0.69;	1.07
Lipoprotein(a)¶ 3.5-fold	0.994	0.90;	1.10	1.04	0.88;	1.22	0.95	0.83;	1.09
Non-HDL-cholesterol, 35 mg/dL	1.28	1.11;	1.42	1.15	0.93;	1.47	1.32	1.11;	1.57
Systolic BP, 23 mmHg	1.35	1.20;	1.54	1.65	1.32;	2.06	1.23	1.05;	1.44
Current vs never smoking	1.08	0.73;	1.61	1.34	0.80;	2.25	0.72	0.34;	1.51
Diabetes, yes/no	2.28	1.37;	3.78	2.64	1.21;	5.73	2.06	1.02;	4.14
Lipid-lowering drugs, yes/no	2.67	1.30;	5.48	1.15	0.16;	8.31	3.51	1.60;	7.73
*No MetS**	70/769††			32/355†			38/414†		
Apolipoprotein A-I, 0.35 g/L	1.32	1.07;	1.68	1.32	0.93;	1.54	1.23	0.87;	1.74
HDL-cholesterol, 0.31 mmol/L	0.88	0.68;	1.13	0.98	0.67;	1.44	0.82	0.58;	1.20
Lipoprotein(a)¶ 3.5-fold	1.03	0.87;	1.23	1.06	0.79;	1.43	1.01	0.80;	1.27
Non-HDL-cholesterol, 35 mg/dL	1.42	1.11;	1.74	1.57	1.11;	1.65	1.37	0.995;	1.87
Systolic BP, 23 mmHg	1.51	1.20;	1.93	1.85	1.23;	2.81	1.44	1.07;	1.93
Current vs never smoking	1.13	0.59;	2.17	1.46	0.59;	3.63	0.64	0.19;	2.22
Diabetes, yes/no	10.1	3.00;	33.9	9.84	2.11;	46.0	12.4	1.55;	99
Lipid-lowering drugs, yes/no	1.97	0.46;	8.40	9.91	1.21;	81.3	1.14	0.15;	9.00
*With MetS**	128/561†			57/252†			71/309†		
Apolipoprotein A-I, 0.35 g/L	1.19	1.04;	1.37	1.19	1.04;	1.42	1.00	0.73;	1.37
HDL-cholesterol, 0.31 mmol/L	0.91	0.73;	1.13	0.83	0.59;	1.17	1.02	0.76;	1.38
Lipoprotein(a)¶ , 3.5-fold	0.98	0.86;	1.12	1.035	0.84;	1.27	0.93	0.78;	1.11
Non-HDL-cholesterol, 35 mg/dL	1.15	0.97;	1.37	0.97	0.73;	1.28	1.28	1.04;	1.63
Systolic BP, 23 mmHg	1.20	1.02;	1.44	1.47	1.07;	2.02	1.10	0.89;	1.38
Current vs never smoking	1.17	0.72;	1.91	1.51	0.78;	2.92	0.75	0.29;	1.92
Diabetes, yes/no	1.64	0.93;	2.90	1.53	0.60;	3.92	1.65	0.77;	3.55
Lipid-lowering drugs	2.50	1.08;	5.80	NS protect. too few			3.92	1.61;	9.58

Models were adjusted also for age (HRs 1.34 to 1.44), and the non-significant variables of (sex) and former smoking

*MetS status at baseline examination.

¶ log-transformed values.

Lipid-lowering drugs were used in 12 subjects without and 23 with MetS (overall in 2.6% of sample, 10 men, 25 women).

Significant values are highlighted in boldface.

†number of cases/number at risk

BP - blood pressure, HDL - high-density lipoprotein, MetS - metabolic syndrome

Discussion

We documented that in a general population apoA-I was, additively to the impaired atheroprotective activity of HDL particles, a CHD risk predictor approaching in magnitude to non HDL-cholesterol or systolic BP in combined sexes when adjusted for conventional risk factors, statin use and Lp(a). Findings provided indirect though sufficient epidemiologic evidence for a hypothesis wherein serum Lp(a) levels aggregated to apoA-I levels to form an autoimmune complex in a milieu of high apoE concentrations and hypertriglyceridemia. C3 levels and use of statin drugs independently contributed in women to the association between high apoE and Lp(a), while the latter's concentration was consistent with apparent inability for immunoassay.

Lp(a) binds oxidized phospholipids and implications for bioassays

Tsimikas and associates (11) documented that plasma levels of oxidized phospholipids present on apo B-100-containing lipoproteins and predominantly on Lp (a) lipoprotein reflect the presence and extent of angiographically documented CAD. They

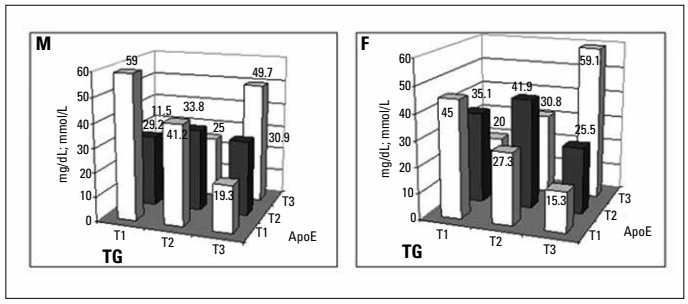

Figure 3. Percentage distribution in 953 men and women is shown of fasting apo E tertiles and triglyceride tertiles, by gender. Measure of agreement, kappa, is highly significant (p<0.001), in men 0.22, in women 0.21. A positive relation is apparent in each gender of the high and low tertiles resulting in high prevalence of both parameters in the highest and lowest tertiles

apo - apolipoprotein

proposed that in settings of enhanced oxidative stress and elevated Lp(a) lipoprotein levels, a proinflammatory milieu may predominate that contributes to clinical cardiovascular disease. In vitro, Lp(a) binds to extracellular matrix proteins such as fibrin and defensins, peptides that are released by neutrophils during inflammation (18).

When Lp(a) aggregates to apoA-I in an autoimmune complex, immunoassays will likely yield an elevated level in apoA-I and a reduced level in Lp(a) commensurate to the portion of Lp(a) protein comprised in the immune complex. Indeed, elevated levels of β2-glycoprotein I-Lp(a) complexes have shown in case-control studies association with coronary artery disease (19, 20). Immunoassay results may be interfered due to failure by capture antibodies to recognize oxidized epitopes interacting with β2-GPI (20)

Recent evidence for apoA-I dysfunction in Western populations

Further to the PREVEND study (13) cited earlier, evidence can be found for the existence of apoA-I dysfunction in Western populations. In the general populations comprised in the meta-analysis by the ERFC (21), apoA-I quintile 2 in over 90,000 individuals (as seen in their Fig. 3) proved to protect against CHD compared with the lowest quintile by nearly 20%. Yet the 3 highest quintiles (across concentrations onwards of 1.44 g/L), conferred protection by no more than 10%. This suggests that intermediate and high apoA-I levels were heterogeneous comprising well-functioning and absolutely dysfunctional apoA-I particles.

The confirmed positive association of serum apoA-I with diabetes in Turkish women was independent of apoE genotype (e2/e3/e4) or of apoB levels (4), and serum apoE concentrations, independent of the apoE genotype, were strongly associated with hypertiglyceridemic dyslipidemias, especially with that accompanying elevated apoB (22, 23).

Reduced Lp(a) levels potentially indicating aggregation in other studies

The poor correlation of Lp(a) values with other variables except apoB or nonHDL-cholesterol (6) can partly be accounted for by its being closely involved in immune complexes. The inverse correlation of Lp(a) with serum fasting triglycerides (6) masks an actual positive correlation between them, potentially precluding the assay ability of Lp(a) during a slow immune response secondary to enhanced low-grade inflammation associated with hypertriglyceridemia. An 11% mean decline in sex- and age-adjusted Lp(a) levels (6) in individuals with diabetes (usually associated with activation of complement pathways) can also be explained by failing to immunoassay part of "damaged" Lp(a) protein and in autoimmune reactivity with apoA-I. Lp(a)'s poor though positive association with CRP and moderate association with fibrinogen are in line with its acute phase reactant features. The fact that HDL-cholesterol and apoA-I were *positively* associated (by a mean 4% and 1%, respectively) with a 1-SD geometric mean Lp(a) increment (6), likely reflects a concomitant process of elevated HDL with proinflammatory elevated Lp(a).

Table 4. Cox regression analysis for incident CHD including metabolic syndrome as an independent variable

	RR	95% CI
	Total	198/1330†
Apolipoprotein A-I, 0.35 g/L	1.23	1.11; 1.37
Presence of MetS	1.86	1.37; 2.52
Lipoprotein(a)¶ 3.5-fold	1.00	0.90; 1.11
Non-HDL-cholesterol, 0.9 mmol/L	1.28	1.11; 1.47
Current vs never smoking	1.11	0.75; 1.63
Diabetes, yes/no	1.90	1.15; 3.15
Lipid-lowering drugs, yes/no	2.89*	1.51; 5.54

*significant in women alone.
†number of cases/number at risk
CHD - coronary heart disease, HDL - high-density lipoprotein, MetS - metabolic syndrome

The fact that plasma Lp(a) decreases dramatically during the third trimester of pregnancy, a state of oxidative stress, while plasma triglycerides increase (24) is consistent with aggregation of Lp(a) in an immune complex. In the postprandial state (25), or after triglyceride infusion (26), a shift to lower densities of apo(a)-containing lipoproteins was observed, and these accumulated when triglyceride levels increased. That Lp(a) and fasting triglycerides are actually positively correlated can be judged also from the effects of the thyroid hormone analogue eprotirome's lowering both lipid variables (27). Interestingly, significant reductions in HDL-cholesterol and especially apoA-I were further notable effects of this drug, suggesting integral improvement of the pro-inflammatory state and HDL dysfunction.

People with smaller apo(a) isoforms have roughly 2-fold higher cardiovascular risk than those with larger proteins (28). It is still unclear whether this association is independent of Lp(a) concentrations, or whether smaller apo(a) isoforms induce susceptibility for elevated circulating pro-inflammatory Lp(a) to attract aggregation to apoA-I particles.

Lp(a)'s role in dysfunction of apoA-I/HDL

The plot of Lp(a) concentrations in the current sample compared with expected ones revealed substantially lacking values exceeding 40 mg/dL and excess values <10 mg/dL, a finding further in support of the current hypothesis of aggregation and "reduced" concentration of elevated Lp(a). Such an aggregation of Lp(a) to apoA-I or another protective protein may modulate the apoA-I particles, elevated in concentrations and pro-inflammatory, ultimately leading to diabetogenicity (1,4) and atherogenicity (2), documented in Turks. The topic of dysfunctional HDL and apoA-I mimetic peptides towards normalization of function has been reviewed (29).

Presence of MetS, and aggregation of Lp(a) to apo A-I

We postulate that during enhanced low-grade inflammation, the Lp-associated phospholipase A_2 contained in Lp(a) cleaves the excessively abundant oxidized phospholipids derived from dietary chylomicrons and remnant lipoproteins bound to their particles and releases short-chain fatty acids and lysolecithin (12). These, in turn, may induce overproduction of VLDL in liver and hypertriglyceridemia and further promote low-grade inflammation. In such a group of subjects (located in the top Lp(a)-tertile and typical in men), high levels of triglycerides likely prevail. Oxidized Lp(a) lipoprotein might aggregate in a moderate degree to apoA-I and/or to apoE which may mediate by the property of avidly presenting lipid antigens (30) in a process in which elevated complement C3 might serve as trigger to the immune signal (31). In an individual without the MetS, atherogenicity of the elevated and dysfunctional apoA-I would be independent of Lp(a) and other established factors, as current findings suggested. In women with MetS, however, excess oxidative stress is associated with substantial immune complex formation, coupled with failure to assay the aggregated Lp(a) protein, whereby the Lp(a)-containing nonHDL-cholesterol assumes predictive ability for CHD risk.

A recent consensus paper recommended Lp(a) levels <50 mg/dL as desirable for the assessment of global cardiovascular risk (12). While levels exceeding this threshold disclose clear tendency to atherogenicity, the present study documents that lower levels may well conceal high CHD risk due to aggregation of Lp(a) (to apoA-I) with consequent development of dysfunctional apoA-I and HDL particles.

Magnitude and threshold of CHD risk for Lp(a) and apoA-I levels

A threshold of Lp(a) is absent regarding excess CHD risk and that a continuous risk association exists across a 100-fold concentration gradient (12). The hazard ratio of multi-adjusted Lp(a) is considered as 1.16 (1.11; 1.2) (6). Yet it may well be argued that concentrations up to about 30 mg/dL, a greater risk is imparted by the associated dysfunctional apoA-I particles, namely, at a magnitude similar to that of conventional risk factors, adjusted for inflammatory biomarkers. The hazard ratio of Lp(a) levels above 30 or 50 mg/dL are recognized as 1.4 (6,12) but might be higher if part of a conferred risk is credited to the presumably aggregated oxidized Lp(a).

Statin use, Lp (a) and cardiometabolic risk

Usage of statin drugs has been reported in meta-analyses (32, 33) to increase the risk of incident diabetes (HR 1.09 (95%CI 1.04; 1.13) (33). A plausible explanation is still needed. We have previously observed such a paradoxical development of diabetes (1, 4), and here we report a positive association between statin usage and CHD incidence in women with (and men without) MetS, independent of the lipoprotein levels. Paradoxical progression of atherosclerosis related to greater LDL-cholesterol reduction was recently reported in subjects using statins combined with ezetemibe (34). Our multivariable linear regression model indicated elevated circulating Lp(a) in females using statin. Turkish women are known to disclose greater pro-inflammatory state, HDL dysfunction and autoimmune activation (3). We have evidence of proteins other than Lp(a) predicting cardiometabolic risk, via involvement in similar autoimmune activation, such as creatinine (35) and thyrotropin.

Clinical implications of apoA-I dysfunction

Impaired function of apoA-I, often associated with dysfunction in HDL particles, is a common phenomenon in the population at large among middle-aged and elderly Turks. Iranian women (36), Australian aborigines, American Indians, and Western people prone to impaired glucose tolerance (13, 37), have been shown or are suspected to disclose HDL dysfunction. The meta-analysis on Lp(a) arouses suspicion that middle-aged Western populations at large may also have inherent HDL dysfunction with respect to CHD risk. Clinicians need to be aware of this possibility with the purpose of correct risk assessment and appropriate measures to be taken. The relevance of Lp(a) to cardiovascular risk is substantially higher than has been hitherto appreciated, since not only the apparent elevated levels but also "reduced" levels due to the immune complex are involved in this risk.

Study limitations and direction of future research

The activity of apoA-I as an independent risk predictor is clearly documented in the current study, though the validity of mediation of Lp(a) to render apoA-I atherogenic is limited by indirect epidemiologic evidence, lacking confirmation by immunochemical analysis. Some analyses were performed in subsets with a comparatively limited sample size. Furthermore, the applicability of present findings to populations with a low prevalence of MetS may be arguable. The relevance of the hypothesis of Lp(a) aggregation to apoA-I in cardiometabolic risk needs confirmation by future research on large sample sizes. The extent of impaired apoA-I function in people prone to impaired glucose tolerance or in general populations as well as its relevance as a constituent of the MetS cluster need to be further investigated.

Conclusion

In a prospective analysis of middle-aged and elderly population sample prone to MetS, this study showed that elevated apoA-I levels acted as a risk factor for CHD, independent of and in a magnitude approaching conventional cardiovascular risk factors. The elicited association was independent also of ATPIII-defined MetS. Epidemiologic evidence was provided that aggregation of the pro-inflammatory Lp(a) to apoA-I in autoimmune activation lay underneath the apoA-I dysfunction, a process which could account for many intricacies of circulating Lp(a), including the apparent inverse relationship to serum triglycerides.

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