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Original article

Serum total and high-density lipoprotein phospholipids: Independent predictive value for cardiometabolic risk

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SUMMARY

Objective: Given that serum phospholipids (PL) may serve as inflammation mediators, we studied whether they predicted metabolic syndrome (MetS), type-2 diabetes or coronary heart disease (CHD) risk in people prone to enhanced low-grade inflammation.
Methods: We analyzed unselected middle-aged Turkish adults with available serum total ($n = 852$) and HDL-PL ($n = 428$) measurements and follow-up (mean 6.6 years) by Cox or logistic regression, after exclusion of prevalent cases of outcome disorder. The enzymatic method used measured total content of phosphatidylcholine, sphingomyelin and lyso-phosphatidylcholine.
Results: Most lipid and non-lipid variables were significantly different in the upper two compared with the lowest total PL tertile, whereby apolipoprotein (apo)A-I and HDL-cholesterol were higher (not lower). ApoA-I, HDL-cholesterol and uric acid were uniformly positive independent linear covariates of total and HDL PL, apoA-I even in participants without MetS. After adjustment for sex, age, waist circumference, HDL-cholesterol and systolic blood pressure, logistic regression for incident MetS disclosed a 3-fold risk (RR [95% CI 1.28; 6.81]) in the upper HDL-pl tertile. In Cox regression models, while the combined two higher HDL-pl tertiles significantly protected against CHD risk in males (HR 0.29 [95% CI 0.10; 0.89]), they weakly tended to impart risk in females: upper two total PL tertiles tended to increased risk of diabetes and CHD.
Conclusion: Excess total PL may mediate inflammatory properties to apoA-I, HDL and uric acid. Excess HDL-pl independently predict risk for MetS in each gender, but are protective against CHD risk in men, possibly because oxidized PL content mediated by total PL is sex-dependent, as reviewed elsewhere.

1. Introduction

Phospholipids (PL) are indispensable in cellular membranes and participate in various enzymatic pathways. Oxidized phospholipids (Ox-PL) can initiate an inflammatory response and are regarded as major pro-inflammatory lipids, next to oxidized low-density lipoprotein (Ox-LDL) known to be critical in the mechanism of atherosclerosis.[1] Tsimikas and associates[2] documented that plasma levels of Ox-PL present on apo B-100-containing lipoproteins and predominantly on lipoprotein [Lp](a) lipoprotein reflect the presence and extent of angiographically documented coronary artery disease. A proinflammatory milieu was proposed to predominate in settings of enhanced oxidative stress and elevated Lp(a) levels that contributed to clinical cardiovascular disease. Lp(a) binds proinflammatory-oxidized phospholipids [2] and is a preferential carrier of oxidized phospholipids (ox-PL) in human plasma. Ox-PL are also present on plasminogen, a homologue of Lp(a), and affect fibrinolysis.[3]

Navab and colleagues[4] proposed a novel hypothesis on the development and reduction of systemic inflammation. Ox-PL and oxidized fatty acids metabolized from arachidonic acid affect endothelial cells and enterocytes in small intestine to induce cytokines influencing macrophages or hepatocytes. The ensuing acute phase reaction needs counteraction by normally functioning high-density lipoproteins (HDL) through its enzymatic activities against inflammation, oxidation and insulin resistance; HDL may be converted to lose its anti-inflammatory properties during a chronic

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acute phase reaction.[4] While among PLs, sphingomyelin is most abundant normally in HDL, increasing phosphatidylcholine (PC) content is associated with adverse clinical conditions.[5] Gut flora has been shown in mice to metabolize dietary PC, a process recently implicated to promote cardiovascular disease.[6]

Turkish adults are susceptible to low-grade inflammation[7] and tend to high risk of coronary heart disease (CHD).[8] Prospective analyses of the cohort of the Turkish Adult Risk Factor study (TARF) demonstrated evidence that dysfunction of serum apolipoprotein (apo) A-I[9] and HDL[10] was a principal factor for the elevated cardiometabolic risk. Cross-sectional analysis of serum total and HDL-PL data in 2004 pointed to an adverse association of total PL (TPL) to metabolic syndrome (MetS)[11] but much more detailed delineation of the relationships was needed.

We report herein results from analysis of the same participants after a follow-up period of up to 8 years. We aimed both to study the predictive value of serum total and HDL-PL for cardiometabolic risk (MetS, type-2 diabetes and CHD) and to better characterize the independent association of the PL with inflammation biomarkers [including Lp(a)] as well as with serum apoA-I and HDL-cholesterol. Finally, a potential adverse association of PL to cardiometabolic risk was explored also in participants without MetS. Findings shed light not only to gender-specificity of independent CHD risk by HDL-pl but also to some aspects of MetS and its relationship to cardiometabolic risk.

2. Methods

2.1. Population sample

The *TARF* 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 scattered communities.[12] It involves a random sample of the Turkish adult population, representatively stratified for sex, age, geographical regions and for rural-urban distribution.

Measurement of serum TPL were made in the 2003/2004 survey of the *Turkish Adult Risk Factor Study*[11] residing in all seven geographical regions of Turkey. HDL-pl were measured in approximately half of the participants (those residing in the Black Sea, East and Southeast Anatolia, Mediterranean and Aegean regions). When 69 participants having no follow-up were excluded, the remaining 852 subjects formed the study sample. The survey was representatively stratified for sex, age, geographic regions and for rural-urban distribution. Informed consent was obtained from each individual, and the survey conformed to the principles embodied in the Declaration of Helsinki. Data were obtained by history of the past years via a questionnaire, physical examination of the cardiovascular system and recording of a resting electrocardiogram (ECG).

2.2. Measurement of risk factors

Waist circumference was measured with the subject standing at the end of gentle expiration at the level midway between the lower rib margin and the iliac crest. Status of cigarette smoking was categorized into current, former and never smokers. Blood pressure (BP) was measured in the seated position on the right arm using an aneroid sphygmomanometer (Erka, Bad Tölz, Germany), after 5 min of rest, and the mean of two recordings was computed. Physical activity was classified as 1) white collar worker, sewing or knitting, 1 km daily walk 2) repair worker 1–2 km daily walk, 3) carpenter, floor and window cleaning, truck driving, 4 km daily walk, 4) heavy work, farming and regular sports activity.

Sera were obtained from venous blood after an overnight >11 h fasting with commercially available kits in a central laboratory in Istanbul. Phospholipids were determined using the phospholipids B kit (Wako Chemicals, Neuss, Germany) adapted to Hitachi 902 autoanalyzer. The method measures phospholipids by their choline content liberated (total phosphatidylcholine, sphingomyelin and lyso-phosphatidylcholine content). The free choline is measured colorimetrically in the presence of choline oxidase and peroxidase.[13] HDL-pl were measured after precipitation of the apoB-containg lipoproteins with phosphotungstic acid-$MgCl_2$. Serum concentrations of total cholesterol, fasting triglycerides, glucose, creatinine and high-density lipoprotein (HDL)-cholesterol (directly without precipitation) were determined using enzymatic kits from Roche Diagnostics (Mannheim, Germany), uric acid by a modified Trinder method (Infinity), and γ-glutamyltransferase (GGT) by Thermo Trace kinetic kit. Concentrations of insulin were measured by chemiluminescence immunoassay utilizing Elecysys 1010 immunautoanalyzer. Apo A-I, apo B, Lp(a), C-reactive protein (hs-CRP) and complement C3 (C3) were measured by means of particle-enhanced immunonephelometry with the Behring nephelometer (Behring Diagnostics). Within run and day to day coefficient of variations for TPL and HDL-pl were <1.1% and 5% for the two level control sera, respectively, and <1.7 and 2.4% for biochemistry parameters on Hitachi,< 3.1 and 4.4% for the blood proteins measured with the nephelometer, respectively, for the two level control sera.

2.3. Definitions

Individuals with *diabetes* were diagnosed with criteria of the American Diabetes Association,[14] namely when plasma fasting glucose was ≥7 mmol/L (or 2-h postprandial glucose >11.1 mmol/L) and/or the current use of diabetes medication. Individuals with *metabolic syndrome* were identified when 3 out of the 5 criteria of the joint conference[15] were met, modified for male abdominal obesity using as cutpoint ≥95 cm, as assessed in the TARF study.[16]

Diagnosis of CHD was based on the presence of angina pectoris, of a history of myocardial infarction with or without accompanying Minnesota codes of the ECG[17] or on 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. CHD death comprised death from heart failure of coronary origin and fatal coronary event.

2.4. Data analysis

Two-sided *t*-tests and Pearson's chi-square tests were used to analyze the differences in means and proportions between groups. Pearson correlation tests were made for continuous variables, including those with log-normalized values. Multiple linear regression analyses were performed with continuous parameters, whereby variables with skewed distribution were log-transformed. The contribution of a significant independent variable as a determinant of phospholipids in a linear regression analysis was calculated by multiplying the related SD value with the β coefficient. Hazard ratio (HR) estimates and 95% confidence intervals (CI) were obtained for diabetes and CHD by use of Cox (and RR for MetS by logistic) regression analyses in models that controlled for potential confounders. Tertiles of phospholipid measures were used as dependent variables in predicting cardiometabolic risk. Sex-specific TPL tertiles were formed by 181 and 209.4 mg/dl in men and 192 and 227.3 mg/dl in women, while corresponding cut-offs for HDL-pl were 33.2 and 41.2 mg/dl in men and 36.1 and 45.2 mg/dl in women. A value of $p < 0.05$ on the two-tail test was considered statistically significant. Statistical analyses were performed using SPSS-10 for Windows (SPSS Inc., Chicago, Ill).

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