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# Predictive value of serum apolipoprotein B/LDL-cholesterol ratio in cardiometabolic risk: Population-based cohort study

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ABSTRACT

**Objectives:** We determined the independent predictive value of serum apolipoprotein (apo) B/LDL-cholesterol ratio for the risk of diabetes, metabolic syndrome (MetS) and coronary heart disease (CHD).

**Design and methods:** Prediction of incident cases was assessed in 2466 adults at 7 years' follow-up.

**Results:** ApoB/LDL ratio was independently associated log-linearly with waist circumference, and, only in men, with HDL-cholesterol in a multivariable regression model. Positive partial correlations existed with fasting insulin, fibrinogen and apo A-I and, only in women, with CRP. Cox regression analyses revealed the two highest apoB/LDL quartiles to be significant determinants of diabetes, at 2-fold RRs, independently of waist circumference, fasting glucose and other confounders. However, apoB/LDL quartiles were not independently associated with CHD in either gender. Only the highest apoB/LDL quartile was associated (RR 1.46) with the development of MetS. Serum apoB/LDL-cholesterol ratio, determined by insulin resistance and in women additionally by pro-inflammatory state, is of independent predictive value for incident diabetes and weakly for MetS, but not for CHD.



## Introduction

Increased level of small, dense low-density lipoprotein (LDL) is part of the atherogenic lipoprotein phenotype (characterized further by decreased HDL-cholesterol and moderately raised triglycerides) that confers elevated risk for cardiovascular disease [1–4]. LDL size has been established as a predictor of cardiovascular events and progression of coronary heart disease (CHD) [5]. Since ultracentrifugation or polyacrylamide gradient gel electrophoresis are required for the determination of LDL particle size and LDL subclass distribution and nuclear magnetic resonance for LDL particle number, clinical surrogates have been sought which involve serum levels of such lipid parameters as triglycerides, apo (apolipoprotein) B, total, LDL- and HDL-cholesterol. Hypertriglyceridemia with elevated apo B [6], an atherogenic dyslipoproteinemia, is the best known surrogate, but total/HDL-cholesterol ratio has also served to reflect LDL particle size [7].

Apo B provides a measure of combined LDL and VLDL particle numbers [6] and is a surrogate measure for increased LDL particle numbers in subjects with MetS and insulin resistance [8,9]. Serum concentrations of apo B are associated with LDL particle number and triglycerides with LDL particle size. Apo B was highly correlated also with LDL-cholesterol of LDL smaller than the median 255 [3]. Smaller LDL particle size is frequently associated with the atherogenic dyslipidemia characterized by MetS [1] and with increased apo B levels [7,10]. The dissociation between LDL-cholesterol and LDL particle number in people with MetS was disclosed in the Framingham study, and increased particle number was found in individuals with MetS for which serum apo B, triglycerides and HDL-cholesterol were best correlates [11]. A higher small LDL particle number was not associated with greater CVD event rates in people with MetS [11]. Authors have considered the possibility whether higher numbers of small LDL particles might still predict cardiovascular risk across a broader spectrum of subjects and in further large samples.

MetS is highly prevalent in Turkish adults [12] who have comparatively high levels of apo B despite fairly low LDL-cholesterol levels [13] and are thus suitable for investigation of the clinical significance of a surrogate of small LDL particles. We, therefore, aimed to study the covariates of the apoB/LDL-cholesterol ratio and its independent predictive values with respect to cardiometabolic risk, including risk of incident MetS, type-2 diabetes and CHD, in a population sample representative of middle-aged male and female Turkish adults.

## Population and methods

### *Population sample*

The *TARF* is a longitudinal population-based cohort study on cardiac disease and its risk factors in adults in Turkey carried out

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biennially in 59 communities in all geographical regions [13]. It involves a random sample of the Turkish adult population, representatively stratified for sex, age, geographical regions and for rural-urban distribution [13]. Combined measurements of waist circumference and HDL-cholesterol having been first made at the follow-up visit in 1997/98, the latter examination formed the baseline. Participants, 28 years of age or older at baseline, were examined periodically up to the survey 2008/09. Excluded was 24% of the cohort with missing concomitant baseline serum values of LDL-cholesterol (including 46 subjects with triglycerides exceeding 400 mg/dl) and apo B so that 2630 participants remained. After the exclusion of 164 individuals with no follow-up, the remaining 2466 participants composed the sample of the current study. The survey conformed to the principles embodied in the Declaration of Helsinki and was approved by the Istanbul University Ethics Committee. Individuals of the cohort gave written consent for participation. 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 12-lead electrocardiogram.

*Measurements of risk variables*

Blood pressure (BP) was measured using a sphygmomanometer (Erka, Bad Tölz, Germany) after 10 min of rest while seated on the right arm, and the mean of two recordings at least 3 min apart was recorded. Waist circumference was measured with a tape (Roche LI95 63B 00), the subject standing and wearing only underwear, at the level midway between the lower rib margin and the iliac crest. Body mass index (BMI) was computed as weight divided by height squared (kg/m [2]). Self-reported cigarette smoking was categorized into never smokers, former smokers (discontinuance of 3 months or more) and current smokers (regularly 1 or more cigarettes daily).

Plasma concentrations of total and HDL cholesterol, fasting triglycerides and glucose were determined at baseline examination by the enzymatic dry chemistry method using a Reflotron apparatus. LDL-cholesterol values were computed according to the Friedewald formula. In the final four surveys, the stated parameters, as well as insulin and C-reactive protein (CRP) values were assayed in serum in a single central laboratory. Blood samples were shipped to Istanbul to be stored in deep-freeze at −75 °C, until analyzed. Concentrations of insulin and sex hormone-binding globulin (SHBG) were determined by the electrochemiluminescence immunoassay ECLIA on Roche Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). Serum concentrations of apo A-I and B, and CRP were measured by the Behring nephelometry (Behring Diagnostics, Marburg, Germany). Fibrinogen levels were assayed in plasma by the modified Clauss method using Behring Fibrintimer II coagulometer and Multifibren U kit. External quality control was performed with a reference laboratory in a random selection of 5–6% of participants. Data on baseline fasting triglycerides, CRP and insulin were available in 74%, 83%, and 56% of participants, respectively.

*Definitions and outcomes*

Individuals with type-2 *diabetes* were diagnosed with criteria of the American Diabetes Association (ADA) [14], namely when plasma fasting glucose was ≥7.0 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 National Cholesterol Education Program (ATP III) [15] were met, modified for prediabetes (fasting glucose 5.6–5.98 mmol/L [16] and further for male abdominal obesity using ≥95 cm as cutpoint [17], as assessed in the Turkish Adult Risk Factor study. The criterion for female abdominal obesity of ≥88 cm was retained [18]. Homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) was calculated in participants who had concomitant fasting insulin and glucose measurements at baseline: HOMA-IR = fasting insulin (μU/ml)* glucose (mmol/l)/22.5 [19].

*Diagnosis of nonfatal CHD* was based on the presence of angina pectoris, of a history of myocardial infarction with or without accompanying Minnesota codes of the ECG [20] 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. Cause of death was assigned in accordance with the information on the mode of death obtained from first-degree relatives and/or local health personnel, considering also pre-existing clinical and laboratory findings elicited during biennial surveys.

*Data analysis*

Descriptive parameters were shown as mean ± standard deviation (SD), in percentages and as adjusted mean ± standard error (SE). Due to skewed distribution, values derived from log-transformed (geometric) means were used for serum CRP, insulin and HOMA. Two-sided *t*-tests and Pearson's chi-square tests were used to analyze differences between means and proportions of two groups; ANOVA was used to detect differences between means of multiple groups, followed by pairwise comparisons with Tukey HSD tests; pairwise comparisons with Bonferroni adjustments were made to detect significance between groups of estimated means. Sex-specific quartiles were formed by following cutoffs: 0.82, 0.9436, 1.141 in men and 0.775, 0.895 and 1.082 in women. Geometric means in the quartiles differed by an average of 1.28-fold higher values so that mean gradient across the top and bottom quartiles were just over 2-fold. In predicting outcomes at baseline examination in multivariate analyses, estimates (and 95% confidence intervals) for hazard ratio (HR) were obtained by use of Cox proportional hazards regression analysis in models that controlled for potential confounders, after exclusion of participants with MetS, diabetes or CHD at baseline examination. HRs for 1 SD of the log-transformed CRP was expressed in terms of a 3-fold increment. A value of $p<0.05$ on the two-sided test was considered statistically significant. Statistical analyses were performed using SPSS-10 for Windows (SPSS Inc., Chicago, Ill., No. 9026510).

## Results

Mean follow-up consisted of 6.2 ± 2.9 years during which 270 cases of incident CHD (19.1 per 1000 person-years) and 216 cases of incident type-2 diabetes (15 per 1000 person-years) were identified, after exclusion of respective prevalent cases. Baseline characteristics of the study population (1167 men and 1299 women, mean age 52.3 ± 11 years) stratified by an apoB/LDL ratio of 0.92 are provided in Table 1. Obesity measures, blood pressure, fibrinogen, serum fasting insulin and triglycerides, were all significantly higher, HDL-cholesterol and SHBG lower in the group with a high apoB/LDL ratio, in which–contrary to anticipation–apo A-I was higher and total cholesterol lower.

Table 2 shows age-controlled partial correlation coefficients of the apoB/LDL ratio with certain variables. Significant correlations existed with most of the variables stated above, while correlations were absent with fasting glucose and physical activity grade and, in men, with smoking status. Gender difference pertained to absent correlations in women with HDL-cholesterol, apo A-I and BMI, while correlation was lacking with CRP in men.

In large subsets of the sample in which serum SHBG, fibrinogen and insulin were available, multiple linear regression analyses were performed to determine covariates of the apoB/LDL ratio. In a model (Table 3) comprising age, waist girth, smoking status, SHBG, HDL-cholesterol and CRP disclosed that waist girth and SHBG were significantly associated with the apoB/LDL ratio in both sexes and