

# Low levels of high density lipoproteins in Turks, a population with elevated hepatic lipase: high density lipoprotein characterization and gender-specific effects of apolipoprotein E genotype

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**Abstract** Turks have strikingly low levels of high density lipoprotein cholesterol (HDL-C) (10–15 mg/dL lower than those of Americans or Western Europeans) associated with elevated hepatic lipase mass and activity. Here we report that Turks have low levels of high density lipoprotein subclass 2 (HDL<sub>2</sub>), apoA-I-containing lipoproteins (LpA-I), and pre- $\beta$ -1 HDL and increased levels of HDL<sub>3</sub> and LpA-I/A-II particles (potentially an atherogenic lipid profile). The frequency distributions of HDL-C and LpA-I levels were skewed toward bimodality in Turkish women but were unimodal in Turkish men. The apoE genotype affected HDL-C and LpA-I levels in women only. In women, but not men, the  $\epsilon$ 2 allele was strikingly more prevalent in those with the highest levels of HDL-C and LpA-I than in those with the lowest levels. The higher prevalence of the  $\epsilon$ 2 allele in these subgroups of women was not explained by plasma triglyceride levels, total cholesterol levels, age, or body mass index. The modulating effects of apoE isoforms on lipolytic hydrolysis of HDL by hepatic lipase (apoE2 preventing efficient hydrolysis) or on lipoprotein receptor binding (apoE2 interacting poorly with the low density lipoprotein receptors) may account for differences in HDL-C levels in Turkish women (the  $\epsilon$ 2 allele being associated with higher HDL levels). In Turkish men, who have substantially higher levels of hepatic lipase activity than women, the modulating effect of apoE may be overwhelmed. **Key words:** The gender-specific impact of the apoE genotype on HDL-C and LpA-I levels in association with elevated levels of hepatic lipase provides new insights into the metabolism of HDL.—Mahley, R. W., J. Pépin, K. Erhan Palaoğlu, M. J. Malloy, J. P. Kane, and T. P. Bersot. Low levels of high density lipoproteins in Turks, a population with elevated hepatic lipase: high density lipoprotein characterization and gender-specific effects of apolipoprotein E genotype. *J. Lipid Res.* 2000. 41: 1290–1301.

**Supplementary key words** HDL<sub>2</sub> • HDL<sub>3</sub> • apoE alleles • apoE genotype • LpA-I • LpA-I/A-II • high density lipoprotein cholesterol • Turkey

In the United States and Western Europe, low levels of high density lipoprotein cholesterol (HDL-C), defined as HDL-C <35 mg/dL, occur in about 15% of men and 5% of women (1). Low HDL-C is associated with an increased prevalence of premature coronary heart disease (2–5). HDL-C levels are influenced by a complex interaction of behavioral, metabolic, and genetic factors. Behavioral factors that impact HDL-C levels include smoking, exercise, obesity, types and amounts of dietary fat, and ethanol consumption (6–12). Metabolic and genetic factors include various primary hypertriglyceridemias, type II diabetes mellitus, isolated low HDL-C, and mutations in the genes encoding apolipoprotein A-I (apoA-I), lecithin:cholesterol acyltransferase, and cholesteryl ester transfer protein (CETP) (5, 13, 14). Variability in the activity of hepatic triglyceride lipase (HTGL) or lipoprotein lipase (LPL) also affects HDL-C levels (13). The apoA-I and HTGL loci account for about one-half of the interindividual variation in HDL-C (15).

Low HDL-C levels are more prevalent in Turkey than in any other country where plasma lipid values have been extensively characterized (16). Data from the Turkish Heart Study have demonstrated that 53% of men and 26% of women in Turkey have HDL-C levels below 35 mg/dL. The low HDL-C levels were found irrespective of geographic location or substantial regional variation in diet, and were not attributable to behavioral factors (smoking, exercise, or alcohol consumption) that commonly modu-

Abbreviations: apo, apolipoprotein; BMI, body mass index; CETP, cholesteryl ester transfer protein; HTGL, hepatic triglyceride lipase; HDL, high density lipoprotein(s); LDL, low density lipoprotein(s); LpA-I, apoA-I-containing lipoproteins; LpA-I/A-II, apo A-I- and apoA-II-containing lipoproteins; LPL, lipoprotein lipase.

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late HDL-C levels. Turks with low HDL-C levels do not have significantly elevated triglyceride levels, suggesting that their low HDL-C levels are predominantly an example of isolated low HDL-C (16). These observations have been confirmed and extended by Onat et al. (17) and Hergenç et al. (18).

In our study to determine the cause of low HDL-C in the Turkish population, the activities of HTGL, LPL, CETP, and lecithin:cholesterol acyltransferase were measured in Turkish men and women residing in Istanbul and compared with the values in non-Turkish control subjects residing in San Francisco (19). In normotriglyceridemic, non-obese subjects, the most striking finding was the elevated HTGL activity in Turkish men and women with reduced mean HDL-C levels (men, 37 mg/dL; women, 43 mg/dL). The mean HTGL activities were 24 and 31% higher in Turkish men and women, respectively, than in age- and sex-matched white Americans with higher mean HDL-C levels (men, 47 mg/dL; women, 58 mg/dL). Elevated HTGL activity is associated with low levels of plasma HDL (13).

In the present study, we characterized in detail the subclasses of plasma HDL in 406 Turkish men and women residing in Istanbul. The HDL were separated into apolipoprotein A-I-containing lipoproteins (LpA-I) and LpA-I/A-II subclasses and HDL subclass 2 (HDL<sub>2</sub>) and HDL<sub>3</sub>. The concentration of pre-β-1 HDL was also determined. Anthropometric data, plasma lipid and lipoprotein cholesterol levels, apoA-I levels, and apoE genotypes were also measured to assess their relationships to the concentrations of the HDL subclasses.

## MATERIALS AND METHODS

### Study subjects

Healthy Turkish volunteers who were employees, or relatives or friends of employees, of the Koç American Hospital in Istanbul were recruited for the study. Subjects with acute or chronic medical problems and those using hypolipidemic drugs, hormone replacement, oral contraceptives, β-blockers, and thiazide diuretics were excluded. There were 196 male and 210 female adult volunteers. The characteristics of the study population are summarized in **Table 1**.

The study protocol was approved by the Committee on Human Research of the University of California, San Francisco, and all participants gave informed consent. All subjects fasted for 12 h before blood was drawn for measurement of plasma lipids and lipoproteins. To prevent coagulation and reduce the possibility of sample degradation before the assay, 10 μL of a solution containing ethylenediaminetetraacetic acid (EDTA; final concentration, 1 mg/mL), chloramphenicol (0.005%), gentamicin sulfate (0.005%), sodium azide (0.01%), and aprotinin (100 IU/mL) was added to each tube. Blood was drawn into precooled tubes and immediately placed on ice. Plasma was separated from the cells within 2 h. Aliquots for the various assays were frozen at -70°C.

### Laboratory procedures

The plasma cholesterol, plasma triglyceride, and HDL-C concentrations were measured by enzymatic techniques in the lipid diagnostic laboratory of the Koç American Hospital. This laboratory has been certified as a lipid reference laboratory by the Cen-

TABLE 1. Characteristics of Istanbul men and women

	Age	BMI	TC	TC >240 mg/dL	LDL-C mg/dL	LDL-C >160 mg/dL	HDL-C mg/dL	HDL-C ≤35 mg/dL	HDL-C >45 mg/dL	TG mg/dL	TG >200 mg/dL	TC/HDL-C	TC/ HDL-C >4.5	TC/ HDL-C >5.5
Men (n = 196)	Mean ± SD 42 ± 12	Mean ± SD 27 ± 4	mg/dL, mean ± SD 197 ± 46	% 16	mg/dL, mean ± SD 126 ± 38	% 17	mg/dL ± SD 36 ± 8	% 56	% 9	mg/dL ± SD 174 ± 106	% 27	Mean ± SD 5.7 ± 1.8	% 72	% 48
Women (n = 210)	Mean ± SD 40 ± 12	Mean ± SD 26 ± 5	mg/dL, mean ± SD 191 ± 50	% 91	mg/dL, mean ± SD 126 ± 42	% 18	mg/dL ± SD 42 ± 9	% 26	% 28	mg/dL ± SD 118 ± 68	% 15	Mean ± SD 4.8 ± 1.6	% 48	% 26

BMI, body mass index; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides.

ters for Disease Control (16). HDL-C levels were measured after very low density lipoproteins (VLDL) and low density lipoproteins (LDL) were precipitated with phosphotungstic acid and magnesium. Kits for the lipid assays were from Boehringer Mannheim (Mannheim, Germany). A Hitachi (Tokyo, Japan) multi-channel analyzer was used for the colorimetric enzymatic determinations (Monotest Cholesterol, CHOD-PAP; Peridochrom Triglyceride, GPO-PAP). The Friedewald calculation was used to estimate LDL-C values (20). Apolipoprotein E phenotyping was performed as described previously (16).

LpA-I were analyzed with an electroimmunoassay based on a previously described immunoassay that had intra- and interassay variations of less than 5% (21). Total apoA-I was measured from whole plasma with Hydrigel apoA-I/B kits (Sebia, Norcross, GA). Plasma was prestained with Sudan black, diluted with the EID buffer provided in the kit, and loaded onto ready-made agarose gels into which monospecific antibodies against apoA-I and apoB had been incorporated. The antibody concentrations were adjusted to produce two rockets on the gels during electrophoresis, one corresponding to apoB and one to apoA-I. Gels were electrophoresed for 3.5 h at 100 V, washed in 0.15 M saline, and dried, and the apoA-I rocket was measured to the nearest half-millimeter. A standard curve was calculated for every gel from standard serum provided in the kit, and sample apoA-I concentrations were calculated from the curve. A control plasma sample was included on every gel.

The HDL<sub>2</sub> and HDL<sub>3</sub> were isolated by double precipitation as described by Gidez et al. (22). All apoB-containing lipoproteins were precipitated by mixing 1 mL of plasma with 0.1 mL of heparin-MnCl<sub>2</sub>. Heparin sodium salt (157 USP units/mg dry weight) and manganese chloride (MnCl<sub>2</sub>·4H<sub>2</sub>O) were obtained from Sigma (St. Louis, MO). The samples were mixed thoroughly, allowed to stand at room temperature for 15 min, and centrifuged at 2,500 rpm for 1 h at 4°C. The supernatant (HDL) was removed. An aliquot was reserved for cholesterol analysis, and the remainder was used to isolate HDL<sub>3</sub>.

HDL<sub>2</sub> were precipitated by adding 0.06 mL of dextran sulfate solution (14.3 mg/dL in 0.15 M NaCl) to 0.6 mL of the heparin-MnCl<sub>2</sub> supernatant. Dextran sulfate (molecular weight 1,500, Dextralip 15) was purchased from Genzyme Diagnostics (Cambridge, MA). The samples were mixed thoroughly by vortexing, incubated at room temperature for 20 min, and centrifuged at 2,500 rpm for 30 min at 4°C. The supernatant (HDL<sub>3</sub>) was removed and reserved for cholesterol analysis.

Total HDL-C and HDL<sub>3</sub>-C in each sample were measured in the same assay with CHOL kits (Boehringer Mannheim) that utilize the CHOD-PAP method. Spectrum system cholesterol standards from Abbott Laboratories (Chicago, IL) were used to calculate a standard curve for each assay. HDL<sub>2</sub>-C was calculated as the difference between total HDL-C and HDL<sub>3</sub>-C.

Pre-β-1 HDL were measured with an isotope dilution assay (23, 24). Total LpA-I was obtained from plasma samples by selective affinity immunosorption, and pre-β-1 HDL were isolated by starch block electrophoresis. The pre-β-1 HDL used for the dilution probe were labeled with tritium and added to the plasma samples. The plasma samples were ultrafiltered at a cutoff of 100 kDa to separate the pre-β-1 HDL from other apoA-I-containing lipoproteins. The specific activity of the ultrafiltrate was determined by scintillation counting, and the apoA-I levels in the plasma and ultrafiltrate were measured by an enzyme-linked immunosorbent assay technique. The amount of pre-β-1 HDL in the plasma was calculated by the isotope dilution equation.

### Statistical analyses

Group means (results listed in the tables) were compared by two-sample *t*-tests. A probability value of 0.05 or less was consid-

ered significant. Relationships between the ε2 allele and levels of HDL-C and LpA-I were assessed by chi-square analysis. Microsoft (Redmond, WA) Excel 5.0 for the Macintosh was used for data management and statistical analyses.

## RESULTS

The study population consisted of 196 Turkish men and 210 Turkish women from Istanbul (Table 1). The men and women were of a similar age and body mass index (BMI) and had similar total cholesterol (~195 mg/dL) and LDL-C (126 mg/dL) levels. In agreement with previous observations (16), the mean HDL-C levels were 10–15 mg/dL lower in both Turkish men and women (36 and 42 mg/dL, respectively) than in U.S. or Western European populations, and ~56% of the men and 26% of the women had HDL-C levels <35 mg/dL. As a result, the mean total cholesterol/HDL-C ratios were markedly higher in Turks than in U.S. subjects (men, 5.7 vs. 4.5; women, 4.8 vs. 3.8) (25). Cholesterol/HDL-C ratios were >4.5 in 70% of Turkish men and 50% of Turkish women, and >5.5 in 48% of the men and 26% of the women. Mean triglyceride levels were higher in men than in women, but were below 175 mg/dL in both groups.

Analysis of the frequency distribution of HDL-C levels (Fig. 1) showed a skewed distribution in Turkish women (a peak at 39 mg/dL and a major shoulder between 44 and 54 mg/dL) and a unimodal distribution without skewedness in men (a peak at 34 mg/dL). In white American men, the unimodal HDL-C distribution, based on NHANES (National Health and Nutrition Examination Surveys) data, peaked at 40–45 mg/dL (26). In American

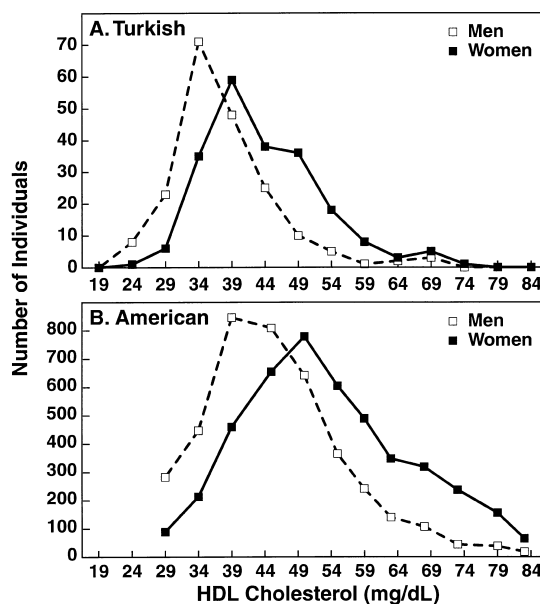


Fig. 1. Frequency distribution of HDL-C levels in Turks (A) and Americans (B). For primary data for U.S. men and women, see ref. 26. The data are plotted as follows: (A) ≤19 = 19, 20–24 = 24, 25–29 = 29, 30–34 = 34 mg/dL, etc.; (B) ≤29 = 29, 30–34 = 34, 35–39 = 39, 40–45 = 49 mg/dL, etc.

TABLE 2. Concentration of lipids (mean mg/dL  $\pm$  SD), apoA-I, LpA-I, and LpA-I/AII (mean mg apoA-I/dL  $\pm$  SD), and ratio of LpA-I:LpA-I/A-II (mean  $\pm$  SD) for normolipidemic subjects of Turkish, French, and American origin

	n	Cholesterol	HDL-C	Total ApoA-I	ApoA-I		
					LpA-I	LpA-I/A-II	LpA-I: LpA-I/A-II
Turkish							
Men	196	197 $\pm$ 46	36 $\pm$ 8	122 $\pm$ 19	39 $\pm$ 9 (31%) <sup>a</sup>	84 $\pm$ 18	0.51 $\pm$ 0.38
Women	210	191 $\pm$ 50	42 $\pm$ 9	123 $\pm$ 19	48 $\pm$ 13 (38%) <sup>a</sup>	76 $\pm$ 18	0.69 $\pm$ 0.33
French <sup>b</sup>							
Men	40	224 $\pm$ 34	59 $\pm$ 15	120 $\pm$ 20	49 $\pm$ 9 (41%) <sup>a</sup>	71 <sup>c</sup>	0.69 <sup>d</sup>
Women	45	208 $\pm$ 36	65 $\pm$ 14	133 $\pm$ 17	60 $\pm$ 13 (45%) <sup>a</sup>	73 <sup>c</sup>	0.82 <sup>d</sup>
American <sup>e</sup>							
Men	27	204 $\pm$ 5	53 $\pm$ 2	123 $\pm$ 4	53 $\pm$ 1 (43%) <sup>a</sup>	70 $\pm$ 3	0.75
Men <sup>f</sup>	49	188 $\pm$ 3	34 $\pm$ 1	102 $\pm$ 3	42 $\pm$ 1 (41%) <sup>a</sup>	60 $\pm$ 3	0.70

<sup>a</sup> Data adapted from ref. 21.

<sup>b</sup> LpA-I as a percentage of total plasma apoA-I.

<sup>c</sup> Calculated (mean total apoA-I – mean apoA-I in LpA-I).

<sup>d</sup> Calculated (mean apoA-I in LpA-I  $\div$  mean apoA-I in LpA-I/A-II).

<sup>e</sup> From ref. 53; reported as mean  $\pm$  SEM.

<sup>f</sup> A low HDL-C subgroup of American men from ref. 53.

women, the HDL-C distribution was also skewed with a peak at  $\sim$ 50 mg/dL and a shoulder at 64 to 74 mg/dL (26).

The HDL in Turks were characterized by electroimmunoassay to measure LpA-I and LpA-I/A-II and by heparin-manganese precipitation to measure HDL<sub>2</sub> and HDL<sub>3</sub>. The plasma apoA-I, LpA-I, and LpA-I/A-II concentrations for Turkish subjects were compared with previously published data from French men and women and American men with normal HDL-C or low HDL-C levels (Table 2). Even though the HDL-C values were 20 mg/dL higher in French and American subjects than in Turks, the plasma total apoA-I levels were similar ( $\sim$ 120–130 mg/dL). However, the Turks had significantly lower LpA-I and higher LpA-I/A-II levels. The ratio of LpA-I to LpA-I/A-II was 0.51 for Turkish men and 0.69 for Turkish women compared with 0.69–0.75 for French and American men and 0.82 for French women. Even in the American men with low HDL-C levels, similar to the typical levels in Turkish men, the LpA-I:LpA-I/A-II ratio was 0.70. The LpA-I accounted for 41–45% of the total plasma apoA-I in non-Turks and 31–38% in the Turks (Table 2).

The frequency distributions of LpA-I levels in Turkish men and women were similar to those of HDL-C levels (Fig. 2). Among men, the distribution was unimodal, with a peak at 40 mg of apoA-I/dL in the LpA-I class. Among women, LpA-I levels peaked at 48 mg/dL, with a shoulder at  $\sim$ 60–70 mg/dL.

For approximately one-fourth of Turkish subjects (selected at random), HDL<sub>2</sub>-C and HDL<sub>3</sub>-C values were compared with LpA-I and LpA-I/A-II values (Table 3). In men and women,  $\sim$ 30–40% of the HDL-C and apoA-I occurred in HDL<sub>2</sub> and LpA-I, respectively, with the remainder in HDL<sub>3</sub> and LpA-I/A-II. LpA-I corresponds roughly

to HDL<sub>2</sub> and LpA-I/A-II to HDL<sub>3</sub> (27–29). HDL<sub>2</sub> and LpA-I levels were lower in subjects with low HDL-C ( $\leq$ 35 mg/dL in men;  $\leq$ 40 mg/dL in women); the difference was especially marked in the women (Table 3). Thus, the low HDL-C levels in Turks were primarily accounted for by reductions in HDL<sub>2</sub>-C and LpA-I levels and a relative increase in HDL<sub>3</sub>-C and LpA-I/A-II levels (Tables 2 and 3).

In a subset of Turks, pre- $\beta$ -1 HDL (the precursor of HDL) values were compared with those of non-Turkish American control subjects living in San Francisco (23). In Turks, the pre- $\beta$ -1 HDL levels were approximately 40% lower than in the controls, and the apoA-I levels were about 10–15% lower (Table 4). Thus, Turks tend to have decreases not only in LpA-I and HDL<sub>2</sub>, but also in pre- $\beta$ -1 HDL precursors.

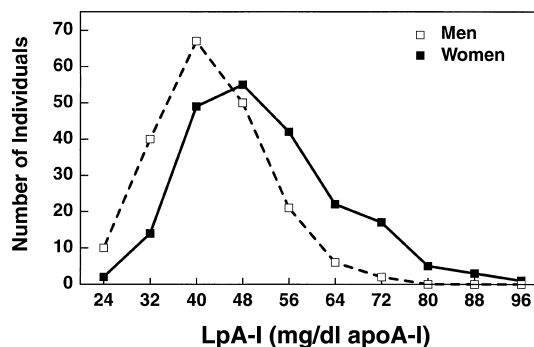


Fig. 2. Frequency distribution of apoA-I in LpA-I particles from Turkish men (open squares) and women (solid squares). The data are plotted as follows:  $\leq$ 24 = 24, 25–32 = 32, 33–40 = 40, 41–48 = 48 mg/dL, etc.

TABLE 3. HDL-C subclasses and LpA-I and LpA-I/A-II values

	n	HDL-C (ppt) <sup>a</sup>	HDL <sub>2</sub> -C (ppt) <sup>a</sup>	HDL <sub>3</sub> -C (ppt) <sup>a</sup>	HDL <sub>2</sub>	HDL <sub>3</sub>	LpA-I	LpA-I/A-II
		mg/dL			% total HDL-C		% total apoA-I	
Turkish men	51	38 ± 9	12 ± 7	26 ± 6	31	69	30	70
Turkish women	49	48 ± 13	18 ± 10	30 ± 6	35	65	40	60
Turkish men								
HDL-C ≤35 mg/dL	26	33 ± 6 <sup>b</sup>	10 ± 5	23 ± 4	30	70	29	71
HDL-C >35 mg/dL	25	43 ± 9 <sup>b</sup>	14 ± 8	28 ± 6	32	68	32	68
Turkish women								
HDL-C ≤40 mg/dL	21	37 ± 7 <sup>c</sup>	10 ± 6 <sup>d</sup>	27 ± 4	27	73	34	66
HDL-C >40 mg/dL	28	57 ± 10 <sup>c</sup>	23 ± 8 <sup>d</sup>	33 ± 7	41	59	45	55

<sup>a</sup> HDL-C determined on fractions after heparin–manganese precipitation.

<sup>b</sup>  $P < 0.01$ .

<sup>c</sup>  $P < 0.001$ .

<sup>d</sup>  $P < 0.005$ .

The effect of increasing triglyceride levels on plasma HDL-C, LpA-I, and LpA-I/A-II is shown in **Table 5**. HDL-C levels decreased significantly as triglyceride levels increased, as previously reported for other populations (30, 31). However, plasma apoA-I levels tended to increase in Turkish men and increased significantly in women as triglyceride levels increased. On the other hand, there was no significant change in LpA-I in men or women, but there was a trend toward higher LpA-I/A-II levels in the men and a significant increase in LpA-I/A-II levels in the women as triglyceride levels increased. Thus, there was a decrease in the LpA-I:LpA-I/A-II ratio with increasing triglyceride levels in the women, but no significant change in the men. Triglycerides showed a modest negative correlation with HDL-C in both Turkish men and women, but not with LpA-I levels (**Fig. 3**).

Because triglyceride levels correlated negatively with HDL-C, we analyzed the effect of increasing HDL-C levels on HDL subclasses in subjects with different triglyceride levels (data not shown). Regardless of whether triglyceride levels were below or above 101 mg/dL in men or women, LpA-I levels increased by 50–60% and apoA-I levels increased by 15–20% as HDL-C increased from <36 to >45 mg/dL. As previously noted (Table 5), at the higher triglyceride levels (comparing triglycerides of <101 vs. >101 mg/dL), there was an increase in total apoA-I.

Our prior study failed to show a significant effect of age on HDL-C levels (16). In the present study, comparing

Turkish men at ages 20–35 versus >50 years, we found that cholesterol and triglyceride levels increased significantly with age (total cholesterol, 176 ± 38 vs. 216 ± 49 mg/dL; triglycerides, 128 ± 69 vs. 202 ± 139 mg/dL), as did the LpA-I levels (36 ± 9 vs. 42 ± 9 mg of apoA-I/dL;  $P = 0.002$ ); however, both HDL-C (36 ± 8 vs. 36 ± 10 mg/dL) and apoA-I levels (118 ± 20 vs. 122 ± 20 mg/dL) remained unchanged. On the other hand, women also showed a significant increase in plasma cholesterol (165 ± 34 vs. 237 ± 52 mg/dL) and triglyceride (81 ± 41 vs. 170 ± 81 mg/dL) levels with increasing age (20–35 vs. >50 years); however, in contrast to the men, women showed significant increases in apoA-I (118 ± 15 vs. 135 ± 21 mg/dL,  $P < 0.001$ ) and in LpA-I/A-II (70 ± 17 vs. 85 ± 19 mg/dL,  $P < 0.001$ ), but no significant change in LpA-I (~50 mg/dL).

Some Turkish women had higher HDL-C levels (44–51 mg/dL) than the majority (39 mg/dL), as shown in Fig. 1. To identify factors that might explain this finding, we examined various anthropometric and biochemical factors (**Table 6**). We focused on BMI and plasma triglyceride levels because of their effects on HDL-C levels. The apoE phenotype was also analyzed because apoE isoforms have differential effects on plasma lipids and because apoE has gender-specific effects (32, 33).

The age distribution and plasma cholesterol levels were similar in Turkish women with low (20–39 mg/dL), medium (40–49 mg/dL), and high (50–75 mg/dL) HDL-C

TABLE 4. Pre-β-1 HDL levels in Americans and Turks

	n	Pre-β-1	HDL-C	ApoA-I	Cholesterol	Triglycerides
		μg/mL apoA-I		mg/dL		
Men						
San Francisco <sup>a</sup>	46	84 ± 49 <sup>b</sup>	48 ± 11	116 ± 27	175 ± 28	81 ± 27
Turkish	17	52 ± 28 <sup>b</sup>	37 ± 7	108 ± 17	169 ± 45	112 ± 33
Women						
San Francisco <sup>a</sup>	90	68 ± 40 <sup>c</sup>	58 ± 12	125 ± 31	179 ± 29	77 ± 23
Turkish	27	38 ± 26 <sup>c</sup>	40 ± 8	113 ± 19	173 ± 19	92 ± 16

<sup>a</sup> Data from O'Connor et al. (23).

<sup>b</sup>  $P < 0.03$ .

<sup>c</sup>  $P < 0.02$ .

TABLE 5. Plasma lipids (mean mg/dL  $\pm$  SD), apoA-I (mean mg/dL  $\pm$  SD), apoA-I in LpA-I (mean mg/dL  $\pm$  SD), apoA-I in LpA-I/A-II (mean mg/dL  $\pm$  SD), and the ratio of apoA-I in LpA-I:LpA-I/A-II (mean  $\pm$  SD) at different triglyceride levels in Turkish men and women

	n	TG	Cholesterol	HDL-C	ApoA-I	ApoA-I		
						LpA-I	LpA-I/A-II	LpA-I: LpA-I/A-II
			mg/dL			mg/dL		
<b>Men</b>								
TG <101	50	76 $\pm$ 16	167 $\pm$ 33	40 $\pm$ 8	119 $\pm$ 18	39 $\pm$ 10	80 $\pm$ 18	0.53 $\pm$ 0.22
TG 101–200	93	148 $\pm$ 30	193 $\pm$ 40	35 $\pm$ 7	120 $\pm$ 20	37 $\pm$ 10	83 $\pm$ 18	0.50 $\pm$ 0.49
TG 201–300	33	249 $\pm$ 31	225 $\pm$ 33	34 $\pm$ 8	127 $\pm$ 21	39 $\pm$ 6	88 $\pm$ 21	0.50 $\pm$ 0.31
TG 301–600	20	417 $\pm$ 87	246 $\pm$ 55	32 $\pm$ 7	129 $\pm$ 17	43 $\pm$ 8	86 $\pm$ 15	0.52 $\pm$ 0.14
<i>P</i> (TG <101 vs. 101–200)			<0.001	<0.001	NS	NS	NS	NS
<i>P</i> (TG <101 vs. 201–300)			<0.001	0.002	0.044	NS	0.042	NS
<i>P</i> (TG <101 vs. 301–600)			<0.001	<0.001	0.025	NS	NS	NS
<b>Women</b>								
TG <101	111	71 $\pm$ 18	166 $\pm$ 35	44 $\pm$ 9	118 $\pm$ 16	50 $\pm$ 13	69 $\pm$ 16	0.79 $\pm$ 0.37
TG 101–200	68	132 $\pm$ 24	204 $\pm$ 44	40 $\pm$ 9	127 $\pm$ 20	46 $\pm$ 13	82 $\pm$ 16	0.58 $\pm$ 0.21
TG 201–300	26	234 $\pm$ 29	250 $\pm$ 42	37 $\pm$ 6	131 $\pm$ 20	44 $\pm$ 10	87 $\pm$ 20	0.56 $\pm$ 0.28
TG 301–600	5	347 $\pm$ 39	265 $\pm$ 60	34 $\pm$ 3	135 $\pm$ 24	46 $\pm$ 11	89 $\pm$ 18	0.52 $\pm$ 0.11
<i>P</i> (TG <101 vs. 101–200)			<0.001	0.003	0.001	NS	<0.001	<0.001
<i>P</i> (TG <101 vs. 201–300)			<0.001	<0.001	<0.001	NS	<0.001	0.004
<i>P</i> (TG <101 vs. 301–600)			<0.001	0.013	0.024	NS	0.007	NS

TG, triglycerides; NS, not significant ( $P > 0.05$ ).

levels (Table 6). However, the BMI and plasma triglyceride levels were significantly higher in the low HDL-C subgroup than in the other subgroups, in which these variables were not significantly different. The apoE allele distributions were strikingly different in the three subgroups (Table 6). The frequency of the  $\epsilon 2$  allele increased progressively by almost sixfold from 1.5 to 5 to 10% in the low, medium, and high HDL-C subgroups, respectively. Similarly, the apoE3/2 phenotype was more frequent at high HDL-C levels (20%) than at medium (11%) or low (3%) levels. The apoE4/3 phenotype was equally distributed in the three subgroups (10, 8, and 11%, respectively). Despite similar age, BMI, and total cholesterol and

triglyceride levels in the medium versus high HDL-C subgroups, the  $\epsilon 2$  allele and the apoE3/2 phenotype frequency approximately doubled as the HDL-C levels increased from 40–49 to 50–75 mg/dL. Thus, these anthropometric and biochemical factors do not explain the increase in the  $\epsilon 2$  allele frequency with higher HDL-C levels in women.

To rule out any association between  $\epsilon 2$  allele frequency and triglyceride levels in the HDL-C subgroups, we analyzed women in the low HDL-C group ( $n = 33$ ; HDL-C = 20–39 mg/dL) and in the high HDL-C group ( $n = 39$ ; HDL-C = 50–75 mg/dL) who had triglyceride levels  $\leq 100$  mg/dL. The two groups were similar in mean age (33 vs. 34 years), BMI ( $24 \pm 4$  vs.  $23 \pm 3$  kg/m<sup>2</sup>), and tri-

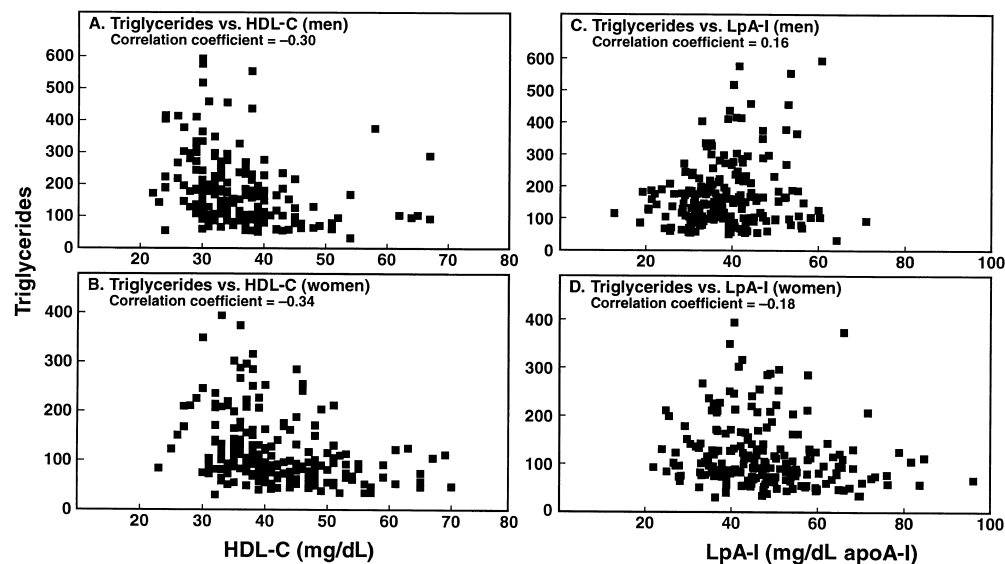


Fig. 3. Relationship between HDL-C levels and triglyceride levels in Turkish men (A) and Turkish women (B). Relationship between apoA-I in LpA-I particles and triglyceride levels in Turkish men (C) and Turkish women (D).

TABLE 6. Characteristics of Turkish women with low, medium, and high HDL-C levels

	HDL-C			<i>P</i>	
	20–39 mg/dL (A)	40–49 mg/dL (B)	50–75 mg/dL (C)	(A) vs. (C)	(B) vs. (C)
n	101	74	35		
Age (years, mean ± SD)	41 ± 11	38 ± 14	38 ± 13	NS	NS
Age >50 years (%)	15	20	20		
BMI (kg/m <sup>2</sup> , mean ± SD)	27 ± 5	24 ± 4	24 ± 6	0.002	NS
TC (mg/dL, mean ± SD)	196 ± 54	182 ± 43	197 ± 49	NS	NS
TG (mg/dL, mean ± SD)	144 ± 76	99 ± 53	82 ± 37	<0.001	NS
Total apoA-I (mg/dL, mean ± SD)	119 ± 18	124 ± 17	135 ± 20	<0.001	0.005
ApoA-I in LpA-I (mg/dL, mean ± SD)	40 ± 9	51 ± 9	62 ± 13	<0.001	<0.001
Total apoA-I in LpA-I (%)	34 ± 8	42 ± 10	46 ± 9	<0.001	0.02
Ratio apoA-I in LpA-I:LpA-I/A-II	0.55 ± 0.22	0.77 ± 0.36	0.92 ± 0.34	<0.001	0.053
Allele frequency (%)					
ε2	1.5	5.5	10.0 <sup>a</sup>		
ε3	93.5	90.4	84.3		
ε4	5.0	4.1	5.7		

TC, total cholesterol; TG, triglycerides; NS, not significant ( $P > 0.05$ ).

<sup>a</sup>  $P < 0.05$  for association of ε2 allele frequency with increasing HDL-C levels (chi-square analysis).

glyceride levels ( $75 \pm 17$  vs.  $66 \pm 19$  mg/dL). However, the higher frequency of the ε2 allele and the apoE3/2 phenotype in the high versus low HDL-C group persisted (ε2, 10 vs. 3%; apoE3/2, 21 vs. 6%).

In the Turkish women, total plasma apoA-I and LpA-I increased as the HDL-C levels increased (Table 6), consistent with the data in Table 3. The increase in apoA-I was associated with higher levels of apoA-I in the LpA-I, which increased from 34 to 46%, and a higher LpA-I:LpA-I/A-II ratio, which increased from 0.55 to 0.92. Analysis of the anthropometric and biochemical factors associated with the skewed distribution of the LpA-I levels in Turkish women (Fig. 2) revealed similar findings (Table 7). The ε2 allele frequency was about threefold higher in subjects with higher LpA-I levels ( $>63$  mg/dL) than in those with low LpA-I levels ( $\leq 50$  mg/dL). Excluding women with triglycerides  $>200$  mg/dL did not significantly alter the distribution of the ε2 allele frequency in the LpA-I subgroups.

The frequencies of the ε2 allele (Table 8) and of the

apoE3/2 phenotype were similar in Turkish men with low and high HDL-C levels. The ε4 allele frequency was slightly higher at the highest HDL-C level; however, the number of subjects was too small to ascertain whether this difference is significant. Nevertheless, Turkish men do not have the prominently skewed HDL-C distribution characteristic of Turkish women (Fig. 1A) and do not show an association of the ε2 allele or apoE3/2 phenotype with higher HDL-C levels.

The distribution of LpA-I values in Turkish men (Fig. 2) was not skewed toward higher values. Nevertheless, we arbitrarily divided the men into three subgroups according to LpA-I. As in the Turkish women, plasma apoA-I levels and the percentage of apoA-I in the LpA-I increased with increasing LpA-I levels (data not shown). However, comparing men and women with the highest LpA-I levels, men had a lower percentage (41 vs. 53%) of apoA-I in the LpA-I and a smaller increase in the LpA-I:LpA-I/A-II ratio. This reflects the fact that as LpA-I levels increased, LpA-I/

TABLE 7. Characteristics of Turkish women with low, medium, and high LpA-I levels

	LpA-I			<i>P</i>	
	≤50 mg/dL (A)	51–63 mg/dL (B)	>63 mg/dL (C)	(A) vs. (C)	(B) vs. (C)
n	134	48	28		
Age (years, mean ± SD)	39 ± 12	39 ± 13	41 ± 15	NS	NS
BMI (kg/m <sup>2</sup> , mean ± SD)	26 ± 5	25 ± 5	24 ± 5	NS	NS
TC (mg/dL, mean ± SD)	190 ± 49	186 ± 47	208 ± 53	NS	NS
TG (mg/dL, mean ± SD)	126 ± 70	107 ± 62	96 ± 65	0.034	NS
Total apoA-I (mg/dL, mean ± SD)	120 ± 17	125 ± 17	137 ± 22	<0.001	0.008
ApoA-I in LpA-I (mg/dL, mean ± SD)	40 ± 7	55 ± 3	71 ± 8	<0.001	<0.001
Total apoA-I in LpA-I (%)	34 ± 7	45 ± 7	53 ± 8	<0.001	<0.001
Ratio apoA-I in LpA-I:LpA-I/A-II	0.53 ± 0.17	0.87 ± 0.30	1.2 ± 0.33	<0.001	<0.001
Allele frequency (%)					
ε2	2.3	8.3	7.1 <sup>a</sup>		
ε3	93.2	84.4	91		
ε4	4.5	7.3	2		

TC, total cholesterol; TG, triglycerides; NS, not significant ( $P > 0.05$ ).

<sup>a</sup>  $P < 0.05$  versus the other two groups (chi-square analysis).

TABLE 8. Characteristics of Turkish men with low, medium, and high HDL-C levels

	HDL-C			<i>P</i>	
	20–31 mg/dL (A)	32–40 mg/dL (B)	41–75 mg/dL (C)	(A) vs. (C)	(B) vs. (C)
n	63	99	34		
Age (years, mean ± SD)	42 ± 11	41 ± 12	42 ± 14	NS	NS
Age >50 years (%)	22	18	29		
BMI (kg/m <sup>2</sup> , mean ± SD)	27 ± 4	26 ± 5	26 ± 4	NS	NS
TC (mg/dL, mean ± SD)	202 ± 49	194 ± 43	194 ± 50	NS	NS
TG (mg/dL, mean ± SD)	224 ± 123	161 ± 89	118 ± 72	<0.001	0.01
Total apoA-I (mg/dL, mean ± SD)	115 ± 19	123 ± 18	133 ± 19	<0.001	0.006
ApoA-I in LpA-I (mg/dL, mean ± SD)	35 ± 9	39 ± 8	45 ± 11	<0.001	<0.001
Total apoA-I in LpA-I (%)	31	32	34	NS	NS
Ratio apoA-I in LpA-I:LpA-I/A-II	0.48 ± 0.26	0.52 ± 0.48	0.55 ± 0.22	NS	NS
Allele frequency (%)					
ε2	7	5	6		
ε3	86	86	81		
ε4	7	9	13		

TC, total cholesterol; TG, triglycerides; NS, not significant ( $P > 0.05$ ).

A-II levels decreased in women but not in men. Also, in contrast to the women, where higher LpA-I levels were associated with lower triglycerides, men with the highest LpA-I values had a significant increase in triglyceride levels (data not shown). Likewise, in contrast to the women, no clear trend was established for ε allele frequency with either low or high LpA-I levels in the men. The ε2, ε3, and ε4 allele frequencies in Turkish men with the high (>46 mg/dL) versus low (≤38 mg/dL) LpA-I levels were 8 versus 5%, 84 versus 88%, and 8 versus 7%, respectively (no significant differences).

## DISCUSSION

Previously we established that Turkish men and women have HDL-C levels that are 10–15 mg/dL lower than those seen in Western European or United States populations (16). Many factors contribute to low HDL-C levels, including obesity, cigarette smoking, physical inactivity, very low fat diets, and various genetic causes (6–14). However, obesity, smoking, physical inactivity, and diet do not explain the low HDL-C levels in Turks (16). Although the subjects in this study had mean BMI values above 25 kg/m<sup>2</sup> (27 ± 4 kg/m<sup>2</sup>, men; 26 ± 5 kg/m<sup>2</sup>, women), a prior analysis of covariance failed to show a significant effect of BMI on HDL-C or LpA-I levels (19). In addition, the same low HDL-C levels seen in Turks living in six different regions of Turkey with quite different dietary habits, are also characteristic of Turks living in Germany (34) and the United States (19). Thus, in all likelihood the low HDL-C levels in Turks are of genetic origin and are not due primarily to secondary environmental factors.

We have investigated several possible genetic causes of low HDL-C in Turks. Most striking in the Turks was a 24–30% elevation in HTGL activity compared with a U.S. control population (19). Even after excluding confounding variables, such as obesity and hypertriglyceridemia, elevated HTGL activity stood out as a distinguishing characteristic of Turkish men and women. The elevated HTGL

activity, which correlated with increased HTGL mass, may reflect an HTGL gene polymorphism or factors independent of the gene itself. The increase in HTGL activity and mass in Turks could be a component of a more generalized genetic and metabolic alteration. For example, Turks tend to have lower LPL levels than American controls; thus, they have an increased HTGL:LPL ratio. Studies are in progress to explore in more detail the genetic basis of the low HDL-C levels seen in the Turkish population.

The present study was undertaken to obtain further insights into the mechanisms responsible for determining HDL levels in a normotriglyceridemic population with low HDL-C levels and elevated HTGL activity. Several important conclusions are suggested by the data generated when HDL are separated into LpA-I and LpA-I/A-II subclasses by electroimmunodiffusion and HDL<sub>2</sub> and HDL<sub>3</sub> subclasses by differential precipitation.

First, Turks have low levels of HDL-C, HDL<sub>2</sub>, and LpA-I. As previously reported (29), the LpA-I correspond roughly to the larger HDL<sub>2</sub>-sized particles, whereas LpA-I/A-II particles correspond roughly to the smaller HDL<sub>3</sub> (27, 28). However, LpA-I can span a range of particle sizes and overlap other HDL subclasses. Low HDL-C levels are associated with increased risk of coronary heart disease, whereas high levels are associated with decreased risk (2–5, 35). More specifically, high levels of HDL<sub>2</sub> are the most protective HDL subclass (36). It has been suggested that LpA-I is cardioprotective, whereas the results from the ECTIM study (37) suggest that both LpA-I and LpA-I/A-II levels predict risk (38). However, for a given plasma apoA-I level in men and women with coronary heart disease, the occurrence of apoA-I in LpA-I rather than in LpA-I/A-II confers greater cardioprotection (39). Likewise, in transgenic mice, apoA-II-containing particles are associated with an increased risk of atherosclerosis (40, 41). As shown in Table 2, Turkish men and women tend to have higher LpA-I/A-II and lower LpA-I levels than French or American subjects. However, total plasma apoA-I levels in these populations are not significantly different, even though HDL-C is 10–20 mg/dL lower in Turks. Thus, the



relative cholesterol content of the LpA-I and LpA-I/A-II particles is lower in Turks. This decrease could reflect a lower concentration of the more cholesterol-rich HDL<sub>2</sub> particles or the effect of higher HTGL activity in remodeling the HDL and promoting CETP-mediated transfer of cholesteryl ester out of the HDL.

Second, pre- $\beta$ -1 HDL levels are ~40% lower in Turkish men and women than in Americans. Pre- $\beta$ -1 HDL, which do not occur in the  $\alpha$ -migrating HDL<sub>2</sub> or HDL<sub>3</sub> ultracentrifugal fractions (42–45), are composed of two molecules of apoA-I (no other apoproteins), and ~10% of their mass consists of phospholipids and unesterified cholesterol (42, 43). Approximately 5–7% of plasma apoA-I resides in pre- $\beta$ -1 HDL. The pre- $\beta$ -1 HDL are the precursors of larger,  $\alpha$ -migrating HDL and can acquire cholesterol from cells (46, 47). Pre- $\beta$ -1 HDL levels correlate positively with plasma apoA-I levels and negatively with HDL-C levels (23). American women have lower levels of pre- $\beta$ -1 HDL than men. Pre- $\beta$ -1 HDL are generated and consumed in a cyclic process (48, 49). Pre- $\beta$ -1 HDL are subsumed into larger,  $\alpha$ -migrating HDL on esterification of their free cholesterol by lecithin:cholesterol acyltransferase. Pre- $\beta$ -1 HDL are then regenerated as cholesteryl esters and are transferred to acceptor lipoproteins by CETP. Both the LpA-I and LpA-I/A-II fractions of HDL can generate pre- $\beta$ -1 HDL in the ester transfer limb of the cycle. The decreased pre- $\beta$ -1 HDL levels in mice overexpressing human lecithin:cholesterol acyltransferase (50) and the high levels in mice overexpressing human CETP (51) are consistent with this cyclic model. Thus, the relative rates of these two processes can influence the steady state levels of pre- $\beta$ -1 HDL in plasma. Changes in the acceptor properties of very low density lipoproteins could also account for reduced levels of pre- $\beta$ -1 HDL by impeding the CETP-mediated transfer of cholesteryl esters. Paradoxically, increased hepatic lipase activity promotes the formation of pre- $\beta$ -1 HDL from HDL<sub>2</sub> (52).

However, the Turks, who are characterized by low HDL-C and high hepatic lipase activity, would be predicted to have high levels of pre- $\beta$ -1 HDL, but in fact they have low pre- $\beta$ -1 HDL levels. These data suggest that because Turks have low HDL levels, the regeneration of pre- $\beta$ -1 HDL by increased hepatic lipase may be impaired. Alternatively, Turks, who have total plasma apoA-I levels similar to those in Americans (53) or French men and women (21), which would also be predictive of high pre- $\beta$ -1 HDL, may have impaired production of apoA-I entering directly into the pre- $\beta$ -1 HDL. It is unclear whether apoA-I can enter the pre- $\beta$ -1 HDL pool by synthesis of these particles by the liver or intestine and/or by generation of apoA-I from remnant lipoproteins during lipolytic processing. The elevated hepatic lipase activity and low HDL-C in the Turks may be part of a metabolic complex characterized by a trend toward higher triglycerides and lower LPL activity (19). Turks have a markedly higher HTGL:LPL ratio than non-Turks, which could fundamentally alter the interrelationships among triglyceride-rich lipoprotein metabolism and HDL subclass metabolism and plasma levels. Therefore, if one source of apoA-I in pre- $\beta$ -1 HDL is from the

surface of lipolyzed remnants and if this pathway is affected by an altered HTGL:LPL ratio, pre- $\beta$ -1 HDL levels may be reduced. It is also interesting that the gender difference in pre- $\beta$ -1 HDL levels in Turks is not significant, whereas American men have significantly higher levels of pre- $\beta$ -1 HDL than American women. Further studies are required to unravel these metabolic pathways.

Third, in Turks, as in other populations (30, 31), higher triglyceride levels were associated with lower HDL-C levels in both men and women. However, the total plasma apoA-I levels increased with increasing triglyceride levels, and the increase in apoA-I was primarily associated with LpA-I/A-II particles. Why does apoA-I increase and HDL-C decrease with increasing triglycerides? The increase in apoA-I is consistent with increased apoA-I production associated with triglyceride-rich lipoproteins and increased generation of apoA-I-rich HDL in the LpA-I/A-II class. The decrease in HDL-C in the face of increased apoA-I-rich particles may reflect the impaired ability of LpA-I/A-II to serve as an acceptor for cholesterol in the reverse cholesterol transport pathway (54) or an increased exchange of triglyceride and cholesterol between very low density lipoproteins and HDL. It is generally agreed that LpA-I particles are better acceptors of cellular cholesterol (55). Whether this is different in the Turks with high levels of HTGL compared with French and American subjects is unknown. However, the decrease in HDL-C and the increase in LpA-I/A-II particles with increasing triglycerides may explain the higher coronary heart disease risk seen in low HDL-C patients with elevated triglyceride levels (56).

Fourth, the higher HDL-C levels in the Turks were associated with higher LpA-I levels and a more favorable LpA-I:LpA-I/A-II ratio in subjects with low triglyceride levels (<101 mg/dL). However, despite increased LpA-I levels with increasing HDL-C, the increase in LpA-I/A-II particles in subjects with higher triglycerides (101–200 mg/dL) decreased the LpA-I:LpA-I/A-II ratio compared with that in subjects with triglycerides <101 mg/dL. If LpA-I/A-II is less capable of reverse cholesterol transport and primarily reflects the HDL<sub>3</sub> subclass, some of the protection afforded by increased HDL-C levels in the Turks may be negated by a higher proportion of LpA-I/A-II particles.

Fifth, the frequency distribution of HDL-C levels was unimodal in Turkish men and was skewed toward bimodality in Turkish women (Fig. 1). A similar difference occurs in Americans. Likewise, the frequency distribution of LpA-I levels was skewed with a significant shoulder in Turkish women and was unimodal in Turkish men (Fig. 2).

Notably, the frequency of the  $\epsilon$ 2 allele in Turkish women increased with HDL-C levels: 1.5, 5.5, and 10% in low (20–39 mg/dL), medium (40–49 mg/dL), and high (50–75 mg/dL) HDL-C subgroups, respectively (Table 7). In a study population of about 10,000 Turks, the distribution of the  $\epsilon$  allele was as follows:  $\epsilon$ 2, 6.1%;  $\epsilon$ 3, 86%; and  $\epsilon$ 4, 7.9% (16). Similarly, the  $\epsilon$ 2 allele was most frequent in Turkish women with the highest LpA-I levels and least frequent in those with the lowest LpA-I levels (Table 7).

Eliminating other factors that might affect the apoE phenotype distribution, such as triglyceride levels or BMI,

did not significantly alter the distribution of the  $\epsilon 2$  allele in the HDL-C and LpA-I subgroups of Turkish women. When subjects with triglycerides  $>100$  mg/dL were excluded, the  $\epsilon 2$  allele was still more frequent in the high HDL-C subgroup. Thus, the  $\epsilon 2$  allele is underrepresented in the low HDL-C and LpA-I subgroups of women and overrepresented in the high HDL-C and LpA-I subgroups. No association of the  $\epsilon 2$  allele with HDL-C and LpA-I levels was seen in Turkish men. In fact, in the men, apoE genotype did not appear to correlate with different HDL-C levels.


Apolipoprotein E polymorphism is one of the most common genetic determinants of interindividual variation in plasma lipid and lipoprotein levels in different populations (57). The  $\epsilon 2$  allele is strongly associated with lower total cholesterol and LDL-C levels, whereas the  $\epsilon 4$  allele is associated with higher levels (58, 59). In the Turkish Heart Study (16), total cholesterol and LDL-C were 11–19 mg/dL lower in both men and women with the apoE3/2 phenotype than in those with the apoE3/3 phenotype, whereas only apoE3/2 males had a significant increase in triglycerides. These results are in general agreement with data from a meta-analysis of populations in 17 countries by Dallongeville et al. (60).

However, various studies have failed to demonstrate a consistent effect of the  $\epsilon$  alleles on HDL-C levels. Dallongeville et al. (60) found no association with the  $\epsilon 2$  allele, but found that the apoE4/3 phenotype was associated with lower HDL-C levels. Importantly, results from the Copenhagen City Heart Study demonstrate gender-specific effects of apoE genotype on HDL-C and apoA-I (61). In Danish women, but not men, the  $\epsilon 2$  allele was associated with higher HDL-C and apoA-I, whereas the  $\epsilon 4$  allele was associated with lower HDL-C and apoA-I levels. In the European Atherosclerosis Research Study (62), the  $\epsilon 2$  allele was associated with higher HDL-C and LpA-I levels. The  $\epsilon 2$  effect was greater in women than in men. In combined data for men and women in the latter study, the  $\epsilon 2$  allele effect was associated with a 3-mg/dL increase in HDL-C levels and a 3.8-mg/dL increase in LpA-I levels.

How could the  $\epsilon 2$  allele be associated with or cause higher HDL-C levels in women? Apolipoprotein E can serve as a cofactor for hepatic lipase, and it especially facilitates the hydrolysis of HDL (63). Because the hierarchy of this stimulatory effect was apoE4  $>$  apoE3  $>$  apoE2, apoE2-containing HDL may be a poorer substrate for HTGL and thus HDL (especially HDL<sub>2</sub>, the preferred substrate for HTGL) could accumulate in the plasma. As shown in Table 6, the higher HDL-C levels were specifically associated with high LpA-I (HDL<sub>2</sub>) levels. Conversely, a decrease in the  $\epsilon 2$  allele associated with HDL could allow the HDL to be a better substrate for HTGL and thus be associated with lower HDL-C levels. In addition, because apoE2 is defective in binding to the LDL receptor (32, 57), apoE2-containing HDL may not be cleared as rapidly from the plasma as apoE3- or apoE4-containing HDL, which bind equally well to the LDL receptor. ApoE has been shown to facilitate the selective uptake of HDL-cholesteryl esters by the scavenger receptor class B type I (SR-BI receptor) (64). It is possible that apoE2 is also de-

fective in facilitating this process. Thus, the  $\epsilon 2$  allele could be associated with increased HDL levels through one or more mechanisms.

Why is the  $\epsilon 2$  effect on HDL-C not seen in men? Although HTGL activity is higher in Turks than Americans, it is lower in women in both populations (19). We speculate that the modulating effect of apoE2 is overwhelmed by the high HTGL activity in men, but not by the lower activity in women. In any case, the HDL-C distribution in women is clearly skewed with a shoulder, which is characterized by higher LpA-I (HDL<sub>2</sub>) levels and a higher frequency of the  $\epsilon 2$  allele.

Because of their isolated low HDL-C levels associated with elevated HTGL mass and activity, the Turkish population is an ideal group in which to explore the genetic basis of low HDL-C. This is important because low HDL-C levels are a common risk factor for accelerated coronary heart disease (2–5). Identification of the association of the  $\epsilon 2$  allele of apoE with higher HDL-C levels in women, but not in men, and the paucity of the  $\epsilon 2$  allele in women with lower HDL-C levels provide additional insights into the factors that affect HDL levels. It will be of interest to study other ethnic groups to determine whether there are similar gender-specific effects of the  $\epsilon 2$  allele of apoE on HDL levels. 

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