Turkish Heart Study: lipids, lipoproteins, and apolipoproteins

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Abstract We examined the plasma lipids, lipoproteins, and selected apolipoproteins in approximately 9,000 men and women from six different regions of Turkey with markedly different diets, ranging from an Aegean coast diet high in olive oil (plasma cholesteryl ester fatty acids enriched in monounsaturated fatty acids) to an inland Anatolian diet high in meat and dairy products (plasma cholesteryl esters enriched in saturated fatty acids). The rural population consuming an olive oil-rich diet had the lowest plasma cholesterol levels (men, 149 mg/dl; women, 150 mg/dl). The urban populations of Istanbul and Adana had higher plasma cholesterol levels (men, 202 and 184 mg/dl, respectively; women, 181 and 190 mg/dl, respectively). Affluent men had the highest cholesterol levels (207 mg/dl). The low density lipoprotein (LDL) cholesterol levels tended to parallel the total cholesterol levels (highest for Istanbul men at 136 mg/dl and lowest for Aegean coast men and women at ~100 mg/dl). Strikingly, the Turkish people were found to have very low levels of high density lipoprotein (HDL) cholesterol (HDL-C) (mean values for all six regions: men, 34–38 mg/dl; women, 37–45 mg/dl) and total cholesterol/HDL-C ratios that were high (mean values for all six regions: men, 4.5–5.5; women, 3.9–5.0). The low HDL-C levels appear to be caused, at least in part, by a genetic factor. Triglyceride levels also tended to be high in Turkish men (~120–150 mg/dl) and women (~90–110 mg/dl). Thus, even though the total plasma cholesterol levels are not excessively elevated in comparison to those in other populations, the presence of low HDL-C or low HDL-C coupled with mildly elevated triglyceride levels may represent a significant risk factor for heart disease in the Turkish population. Affluence and higher education were associated with higher cholesterol levels. Lack of physical activity, smoking, and alcohol consumption also tended to be associated with a detrimental lipid profile. Lipoprotein[a] levels were identical among the regions surveyed (mean: 11–15 mg/dl) and displayed the typical distribution with an increased number of individuals with low levels. The 90th percentile value for lipoprotein[a] was about 30 mg/dl for both men and women. Smoking, a major risk factor for heart disease, was very prevalent in the Turkish population, especially in men (50–70% smokers) and women in urban areas (30–40% smokers). Hypertension, defined as a systolic pressure of >140 or a diastolic pressure of >90 occurred in ~17% and 26% of the men and women surveyed, respectively. Apolipoprotein (apo) E phenotyping of more than 8,000 individuals demonstrated that the Turks differed from U.S. and European populations, having a higher prevalence of the ε3 allele and a lower prevalence of the ε2 and ε4 alleles. In general, the ε2 allele was associated with lower total cholesterol and LDL cholesterol levels. However, the ε4 allele had only a limited effect (if any) in elevating plasma cholesterol and LDL cholesterol levels in men. On the other hand, apoE4 homozygosity had a significant effect in elevating cholesterol levels in women. Apolipoprotein E2 homozygosity was associated with markedly elevated triglyceride levels (increased by 116 mg/dl) in men; however, apoE3/2 men (but not women) had a mild elevation (+11 mg/dl). Interestingly, apoE4/3 men and women also had an increase in triglyceride levels (+11 mg/dl and +14 mg/dl, respectively, compared to apoE3/3), but apoE4/4 homozygotes had no significant effect on triglyceride levels. Furthermore, the ε2 and ε4 alleles were not associated with significant differences in HDL-C levels in the Turkish population. Unlike the U.S. and European populations, where the 3500 mutation of apoB-100 that is responsible for familial defective apoB is rather common, this mutation was not found in the Turkish population.—**Mahley, R. W., K. E. Palaoğlu, Z. Atak, J. Dawson-Pepin, A-M. Langlois, V. Cheung, H. Onat, P. Fulks, L. L. Mahley, F. Vakar, S. Özbayrakçı, O. Gökdemir, and W. Winkler.** Turkish Heart Study: lipids, lipoproteins, and apolipoproteins. *J. Lipid Res.* 1995. **36:** 839–859.

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Numerous studies have established the predictive power of plasma cholesterol levels as a major risk factor for coronary artery disease (CAD) in various populations (for review, see refs. 1–5). In addition, elevated triglyceride levels have been correlated with the increased incidence of CAD in some but not all studies (for review, see refs. 1, 6–8). Furthermore, a low level of high density lipoprotein

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoproteins; CAD, coronary artery disease; FDB, familial defective apolipoprotein B-100; HDL-C, high density lipoprotein cholesterol; Lp[a], lipoprotein[a]; LDL-C, low density lipoprotein cholesterol.

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cholesterol (HDL-C) has been found to correlate strongly with CAD, whereas high levels of HDL-C appear to be protective (1, 9–14). High levels of lipoprotein[a] (Lp[a]) also have been found to correlate with risk of CAD in many (for review, see refs. 15–17) but not all studies (18, 19). The Turkish Heart Study was undertaken to characterize the lipid and lipoprotein variables of the Turkish population. Turkey, which has a high incidence of CAD (estimated at 37% of deaths by the Turkish Ministry of Health), is unique in the diversity of diets consumed within distinct regions of the country. This diversity extends from an Aegean coast diet that is very high in olive oil to a saturated fat-rich diet in central Anatolia (the Asian portion of Turkey). Little detailed lipid and lipoprotein data are available from Turkey, except for an important recent report on triglyceride and cholesterol levels in plasma, obtained by the fingerstick method using the Reflotron apparatus, on 3,689 individuals from 59 communities in different regions of Turkey (20).

The present study also was designed to examine the impact of the isoforms of apolipoprotein (apo) E on lipid levels and to screen for the apoB mutation responsible for familial defective apoB-100 (FDB) in the Turkish population. The common isoforms of apoE (E2, E3, and E4) influence lipid levels and lipoprotein metabolism (for review, see refs. 21 and 22). For example, it has been shown that apoE2 is associated with decreased levels of plasma cholesterol, whereas the opposite occurs with apoE4 (21–24). For a single gene apoE has an unusually large effect on plasma cholesterol levels and may account for about 10% of the interindividual variation in different populations. The mutation in apoB-100 involving a glutamine substitution for arginine at residue 3500 has been shown to be commonly associated with increased total cholesterol and low density lipoprotein cholesterol (LDL-C) levels in certain populations (for review, see ref. 25). Therefore, the prevalences of the common apoE isoforms and of FDB have been ascertained to determine the influence of these factors on plasma cholesterol and LDL-C levels in the Turkish population.

Characterization of the Turkish population provides knowledge of the factors implicated in heart disease risk in a part of the world seldom studied. The Turkish Heart Study identified important genetic and environmental factors that impact plasma lipids and lipoproteins, highlighted individuals at increased risk of heart disease in a country of 60 million people, and provided baseline data for future studies.

MATERIALS AND METHODS

Study design

Six diverse regions of Turkey were selected for this cross-sectional survey. In each region, men and women were invited to participate as volunteers in the Turkish Heart Study by coming to a clinic site fasting (abstinence from eating for more than 12 h) to have a 10-ml blood sample (0.1% EDTA) drawn and to have a personal interview conducted. No incentives for participation in the study were provided other than the importance of being part of a national survey. Blood samples were immediately placed on ice, and plasma aliquots and buffy coats were prepared within approximately 4–5 h after phlebotomy, frozen in dry ice, and stored at −30°C to −70°C until analyzed. Health survey interviews were conducted by participating Turkish physicians and medical students who had been trained to fill out the biodata form and who received a 16-h lecture course on lipids and atherogenesis.

Regions surveyed

Approximately 9,000 volunteer participants were enrolled during 1990 through 1993. The six regions and their characteristics are as follows (**Fig. 1**).

1. Istanbul is a large, modern, metropolitan city with a population of approximately 9 million. It has grown rapidly over the past 10 years with the migration of rural Turkish people to the city. The Istanbul participants included management/executive personnel from several Turkish companies who came for routine physical examinations to the checkup clinic at the American Hospital located in Istanbul. In addition, the participants included factory, hospital, and office workers within the city of Istanbul.

2. Adana is a city of 1.2 million in the southeastern part of Turkey near the Syrian border. Its people are known to consume a diet high in lamb. Factory employees, business people, and professionals were surveyed. Rotary Club members and their families participated in the study as volunteers and solicited participants among other business people in the city.

3. The region of Trabzon included participants in the city of Vakfikebir (population, 25,000) and numerous small towns (Tonya) and villages in the northeastern region of Turkey near the Black Sea. This region is noted for the consumption of cheese and other dairy products. Town and village participants were primarily involved in agricultural work, whereas participants living in Vakfikebir were business people and professionals.

4. Kayseri, a city of ~500,000 people, is located in central Turkey. The participants were from the city and surrounding villages and included factory workers, agricultural workers, and business/professional people. The region is noted for the consumption of meat and dairy products. Rotary and Lions Club members and their families participated in the study.

5. Aydin (population of 120,000) is located in southwestern Turkey in a region where sunflower and corn oils are produced and consumed. The population surveyed included factory workers and business/professional people

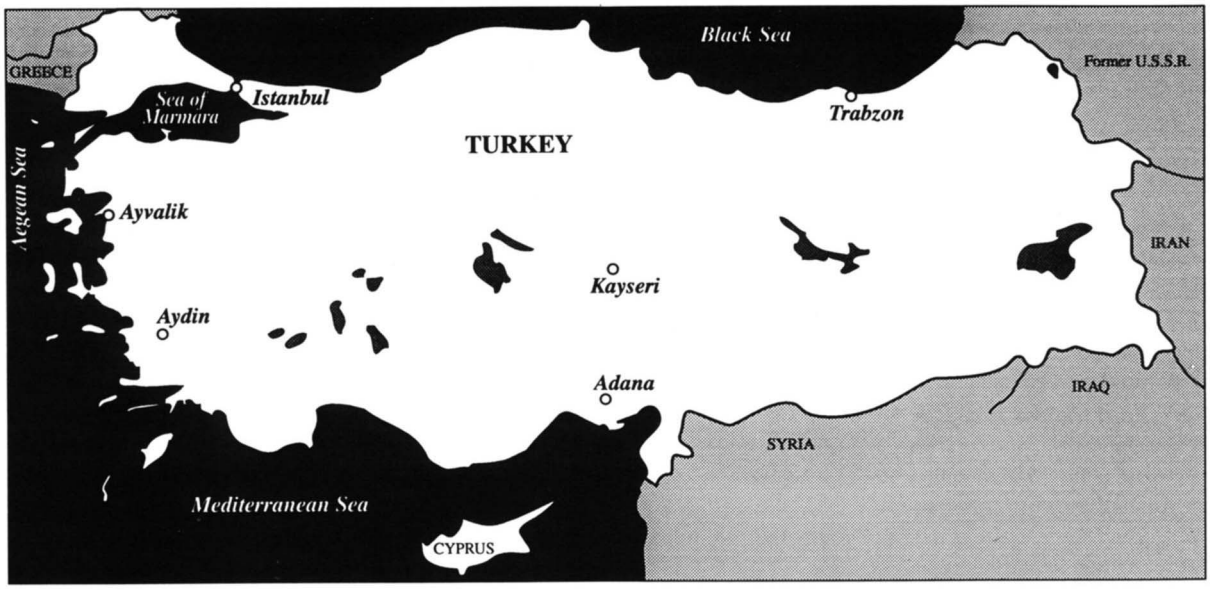

Fig. 1. Map of Turkey showing the approximate locations of the regions surveyed in the Turkish Heart Study.

living in and around Aydin. Members of the Rotary Club also participated in the study.

6. Ayvalik is a small city of 50,000 people on the Aegean Sea in a region noted for the growing of olives and the production of olive oil. The participants were village people primarily engaged in agriculture and business/professional people living in the city of Ayvalik. Rotary Club members participated as volunteers and recruited participants.

Biochemical analyses

A diagnostic lipid laboratory was established at the American Hospital (Istanbul) to measure total cholesterol, triglycerides, and HDL-C. The laboratory was certified as a reference laboratory by the Lipid Research Center of the Baylor-Methodist Hospital (coordinated by Dr. Wolfgang Patsch) in Houston, TX. The LDL-C was calculated using the Friedewald formula (26) for participants with triglyceride levels <400 mg/dl. All reagent kits were provided by Boehringer-Mannheim (Mannheim, Germany). Total plasma cholesterol and triglyceride levels were determined on a Hitachi multianalyzer using enzymatic colorimetric methods (Monotest Cholesterol, CHOD-PAP; Peridochrom Triglyceride, GPO-PAP). The HDL-C levels were determined, after phosphotungstic acid–magnesium precipitation of very low density lipoproteins (VLDL) and LDL, using the CHOD-PAP method. Lipoprotein[a] levels were determined using the sequential sandwich ELISA method (Macra Lp[a], Strategic Diagnostics, Newark, DE). Samples were selected from each survey population for analysis and reported as mg of Lp[a]/dl.

Apolipoprotein E phenotyping was performed by isoelectric focusing of delipidated serum by the method of Menzel and Utermann (27). The proteins were transferred to nitrocellulose paper and immunostained. The primary antibody was prepared in a rabbit against human apoE (gift from Karl H. Weisgraber). The secondary antibody was a biotinylated anti-rabbit IgG (Vector Laboratories, Inc., Burlingame, CA). The immunoblot, incubated with avidin and biotin (Vectastain ABC Rabbit IgG Kit, Vector Laboratories, Inc.), was stained with diaminobenzoic acid and the color was developed with hydrogen peroxide. The phenotype was assigned by consensus of two or three independent reviewers. Discrepancies ($<10\%$ of the samples) were resolved by repeating the isoelectric focusing. Genotyping (described below) was used to confirm results where the phenotype was difficult to assign.

Apolipoprotein E genotyping was performed by gene amplification and cleavage by the *Hha*I restriction enzyme, as described by Hixson and Vernier (28). The DNA from frozen buffy coats was extracted using a lysis buffer described by Buffone and Darlington (29) followed by polymerase chain reaction amplification. The reaction mixture was separated on polyacrylamide gels and the products were visualized by ethidium bromide staining and UV transillumination. Genotyping was performed on all samples from subjects with plasma cholesterol levels ≥ 250 mg/dl and on all samples demonstrating at least one apoE2 or apoE4 isoform by phenotyping. Genotype assignment was established by consensus of two or three independent reviewers, and ambiguous assignment of genotype resulted in a repeat analysis. Phenotype and genotype assignments were in agreement $\sim 98.5\%$ of the time. The mismatches are the subject of future studies.

The phenotype results were used for data analyses reported in this study.

Screening for the Arg→Gln substitution at residue 3500 of apoB-100 was performed on DNA extracted from frozen buffy coats using the lysis buffer described above. The apoB 3500 mutation (CGG→CAG) was detected using gene amplification and cleavage with *Msp*I as described by Hansen et al. (30). The reaction products were visualized by ethidium bromide staining and UV transillumination. The assay was conducted on all samples from individuals with plasma cholesterol levels ≥220 mg/dl and on other samples selected from each regional population without regard for lipid levels or other parameters (tubes were drawn randomly from freezer bags containing samples from the different regions).

Fatty acid analyses of plasma cholesteryl esters were performed as follows. Plasma (300 μl) was freeze-dried and extracted with 2 ml of chloroform–methanol 2:1. The volume of the extract was reduced and the extract was applied to a thin-layer chromatography plate (ANALTECH silica gel G 250 microns) that was pre-washed with methanol and heat-activated (100°C for 15 min). The plate was developed in a system of hexane–diethyl ether–concentrated ammonium hydroxide 80:20:1 and the lipids were visualized with iodine vapor. Cholesteryl ester bands were identified by comparison to standards, marked, and, after sublimation of the iodine, scraped from the plate.

Transesterification was performed in the presence of the silica gel by incubation overnight at 37°C with 1 ml of a mixture of 3 N methanolic·HCl (Supelco, Inc., Bellefonte, PA) and toluene (4:1). Methyl esters were extracted by adding 1 ml hexane and 0.5 ml H_2O, vortexing, and recovering the hexane layer. The methyl esters derived from the cholesteryl esters were purified by thin-layer chromatography (as described above) to remove free cholesterol and extracted from the silica gel with chloroform–methanol 2:1. The fatty acid methyl esters were analyzed using a Hewlett Packard 5880A gas chromatograph using a Supelco 10% SP-2330 packed column. The initial temperature of 175°C was maintained for 5 min and then increased at 2.5°C/min, until a final temperature of 210°C was attained. The samples analyzed for cholesteryl ester fatty acids were drawn randomly from each regional population without regard for lipid values or other parameters.

Personal and clinical data obtained at the time of the interview

Personal data (age, sex, weight, height, and blood pressure) were obtained from all participants. Blood pressure measurements (mm Hg) were determined while the participants were seated. In addition, a questionnaire with approximately 30 questions was designed to obtain relevant social (marital status, profession, salary, smoking, alcohol consumption, physical activity), medical (general health, cardiovascular health, physician visits), and family (general and cardiovascular health of parents and siblings) history. Many of these variables will be evaluated in more detail in future studies.

The following variables, as ascertained by the biodata questionnaire, were found to be associated with variations in the different lipid levels determined in this study. Salary was assessed by asking the participant to estimate the total monthly income for the family unit living together. Four categories were listed, with the lowest salary equivalent to less than $200/month and the highest salary equivalent to greater than $1,000/month. The value for the Turkish lira was adjusted approximately every 6 months on the basis of the valuation of the U.S. dollar. The levels of education included the following categories: none, primary school, middle school, high school, university, and postgraduate degree program. Smoking was assessed by asking how many cigarettes were smoked per day. Alcohol intake was determined irrespective of the type of alcohol consumed (beer, wine, or hard liquor) and recorded in one of three categories: none or very little per week, 1–5 glasses per week, and greater than 5 glasses per week. The type of work, as a measure of physical activity, was determined on the basis of three general categories: physical/active work (high energy expenditure), office/shop work, and administrative/management work.

Data entry and statistical analysis

Questionnaire development and data collection were accomplished with *Epi Info Version 5.0* (31). The prevalence of selected categorical variables and mean levels of continuous variables were calculated utilizing the same software. Risk factors analyzed as continuous variables included age, body mass index, total cholesterol, LDL-C, HDL-C, triglycerides, Lp[a], plasma cholesteryl ester fatty acids, and the ratio of LDL-C to HDL-C. Salary, education, type of work, smoking, and alcohol consumption were analyzed as categorical variables. Statistical significance of differences between the mean lipid levels of the various subgroups was determined by utilizing ANOVA for variables with normal distributions and the Kruskal Wallis test for variables not normally distributed (32). Where indicated, mean lipid and lipoprotein values were adjusted for age (a study design variable). These adjustments were made using the PROC GLM procedure in *SAS* (33).

RESULTS

Characterization of the various study populations

The number and general characteristics of the participants in each region are summarized in **Table 1.** The participants ranged in age from 20 to 100 years (97.5% were 70 years or younger), but the mean ages in each

TABLE 1. Characterization of the various study populations surveyed

Location	Total		Age[a]		Systolic BP >140 or Diastolic BP >90		Percent of Smokers		Body Mass Index[a]		Weight[a]	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
			yr		%		%		*kg/m²*		*kg*	
All	6085	2932	40.4 (12.2)	41.8 (14.8)	16.7	26.0	55.3	19.3	25.6 (3.7)	26.0 (5.3)	74.4 (11.9)	63.3 (12.7)
Istanbul	2147	523	39.8	31.9	10.6	5.3	49.3	42.7	25.6	23.7	77.7	60.8
Adana	1089	207	36.1	38.5	19.6	21.4	58.3	31.9	26.8	27.1	77.4	68.1
Trabzon	731	748	50.0	45.9	31.9	32.7	46.4	3.9	24.5	25.2	69.1	59.2
Kayseri	721	506	41.0	45.7	18.9	38.6	60.4	6.7	25.1	28.5	70.9	67.4
Aydin	674	400	37.0	35.2	9.2	12.8	72.6	27.8	25.5	26.1	73.9	65.4
Ayvalik	601	535	47.0	48.4	29.0	36.9	56.7	18.6	25.3	26.7	71.3	64.0
Other[b]	122	13	42.5	42.0	11.5	30.8	50.8	30.8	26.4	26.3	79.4	68.8

[a]Mean (SD).
[b]Includes men and women who were not from the region being surveyed but who asked to participate in the study.

regional population tended to be between 35 and 50 years of age. Hypertension, described as either a systolic pressure >140 or a diastolic pressure >90, was common and variable from region to region. The women appeared to have a higher prevalence of hypertension, except in Istanbul. The blood pressure measurements represent a single reading taken at the time of the personal interview. The women in the various regions had a higher mean body mass index (kg/m²) than the men, except for women in Istanbul. Smoking, presented as the percentage of current smokers, is widespread in Turkish men and highly variable in women (Table 1). In the more conservative areas (Trabzon region and Kayseri) the women were less likely to smoke. The null hypothesis of homogeneity among regions was rejected ($P < 0.000001$) for all traits listed in Table 1; however, the role that sampling bias may have played in these dissimilarities cannot be ruled out. Further detailed analyses of these and other characteristics of the population will be presented in future reports.

As shown in **Table 2,** a sample from each population was analyzed for fatty acid content of the plasma cholesteryl esters. Striking regional differences were observed (Table 2). The values obtained in the Ayvalik and Aydin populations reflect the relatively high levels of monounsaturated (olive oil) and polyunsaturated (sunflower and corn oils) fats consumed in these regions, respectively. The cholesteryl ester fatty acids in the samples from among the volunteers in the Trabzon region, Kayseri, and Adana were enriched in saturated fatty acids compared to the values obtained for samples from Aydin and Ayvalik, and reflect the high consumption of meat (primarily lamb) and dairy products (Table 2). In Istanbul the diet is more varied than in other regions, with an abundance of processed foods and oils available, and this diversity is reflected in the fatty acid analyses of this population. De-

TABLE 2. Fatty acid analysis of plasma cholesteryl esters by region (% of total fatty acids)[a]

Fatty Acids	Istanbul	Adana	Trabzon	Kayseri	Aydin	Ayvalik	*P*
C14:0	0.53 (0.33)	0.60 (0.44)	2.0 (1.3)	0.69 (0.32)	0.36 (0.21)	0.35 (0.27)	[b]
C16:0	16.8 (4.5)	20.0 (3.5)	18.8 (3.4)	20.4 (2.8)	14.0 (2.7)	15.1 (3.0)	[b]
C18:0	2.3 (1.6)	3.2 (2.1)	4.1 (2.1)	3.0 (1.2)	1.3 (1.0)	1.7 (1.5)	[b]
C18:1	20.3 (2.9)	18.7 (2.7)	21.9 (3.1)	21.9 (3.5)	20.0 (4.3)	28.8 (4.4)	[b]
C18:2	49.6 (6.5)	48.9 (6.0)	39.8 (6.4)	44.1 (5.9)	53.8 (6.6)	44.4 (6.0)	[b]
Saturated[f]	19.6 (6.0)	23.8 (5.2)	24.9 (5.0)	24.1 (3.6)	15.7 (3.4)	17.1 (4.1)	[c]
Monounsaturated[g]	23.6 (3.4)	21.6 (3.1)	27.3 (4.0)	25.8 (4.0)	22.7 (5.0)	31.6 (4.7)	[d]
Polyunsaturated[h]	56.4 (8.1)	54.4 (5.9)	46.9 (6.9)	49.8 (6.1)	60.9 (7.1)	50.8 (6.4)	[e]
n	109	141	92	65	123	129	

[a]Mean (SD).
[b]Significance established between the highest and lowest value reported among the populations ($P < 0.001$).
[c]Adana, Trabzon, and Kayseri samples have significantly higher saturated fatty acids than those from Istanbul ($P < 0.001$). Istanbul samples have higher levels of saturated fatty acids than observed for the samples from Aydin and Ayvalik ($P < 0.001$).
[d]Ayvalik samples have significantly higher monounsaturated fatty acids compared to those from all other locations ($P < 0.001$).
[e]Aydin samples have significantly higher polyunsaturated fatty acids compared to those from all other locations ($P < 0.001$).
[f]Includes C14:0, C16:0, and C18:0.
[g]Includes C16:1 and C18:1.
[h]Includes C18:2, C18:3, C20:3, and C20:4.

tails of the fatty acid analyses from subgroups within the Istanbul population will be presented later in this report.

Plasma lipid and lipoprotein levels in men and women in the various regions

Age-adjusted plasma cholesterol levels demonstrated that the mean values were the highest for men in Istanbul and the lowest for men and women in Ayvalik (**Table 3**). The women in Adana, Trabzon, and Kayseri tended to have higher mean levels than did the men of these same populations, and women in Aydin and Ayvalik had the lowest cholesterol levels. As shown in Table 3, LDL-C levels tended to parallel plasma cholesterol levels. The LDL-C levels were the lowest in Aydin and Ayvalik for both men and women and highest in men from Istanbul. However, HDL-C levels in all regions tended to be lower for both men and women (Table 3) than has been reported for U.S. and European populations (34, 35). The total cholesterol/HDL-C ratios reflected the relatively low level of HDL-C. Men in all regions had mean ratios of 4.5 or greater, and the lowest ratios occurred in the male populations of Aydin and Ayvalik. For women, the ratios ranged from approximately 4 to 5, with the lowest ratios occurring in the women of Aydin, Ayvalik, and Istanbul. It has been emphasized that a cholesterol/HDL-C ratio of 3.5 or less is desirable and that a ratio of 4.5 or greater is considered to be high-risk (36–38). In all regions triglyceride levels were higher in men than in women (Table 3). It is apparent that the "other" category listed in Table 3 was self-selected for high plasma lipids. Individuals in this category were volunteers who were not from the region being surveyed but who were visiting the region and asked to participate. They were not included in any of the analyses to follow and represent a very small percentage of the population surveyed (122 men and 13 women).

Plasma cholesterol levels. Approximately two-thirds of the men and three-fourths of the women (all regions combined) had plasma cholesterol levels of less than 200 mg/dl (**Table 4**). Only 10% of the men and 6% of the women had levels ≥240 mg/dl. However, the populations of the various regions displayed interesting differences. Plasma cholesterol levels in excess of 200 mg/dl occurred in 48% of the male population surveyed in Istanbul, where the mean cholesterol level was the highest. In addition, ~20% of Istanbul men had plasma cholesterol levels ≥240 mg/dl. This percentage is identical to that of the U.S. male population with total cholesterol levels ≥240 mg/dl (~20% in the NHANES III study, 1988–1991) (39). Istanbul women had less hypercholesterolemia and, in all the other survey regions, less than 10% of men and women had cholesterol levels ≥240 mg/dl. The lowest cholesterol levels were in the men and women of Aydin and Ayvalik.

Figure 2A illustrates the relationship between age and plasma cholesterol levels in men and women and is plotted as the mean value for all the regions. Included for comparison are the values obtained for Istanbul men and women plotted separately. Similar results were obtained in the study performed by the Turkish Society of Cardiology on 3,689 individuals in 59 communities throughout Turkey using the Reflotron methodology (20).

Plasma LDL-C levels. As summarized in Table 4, 63% of the men and 72% of the women had LDL-C levels <130 mg/dl; however, the distribution of LDL-C varied from region to region. For example, 53% of the men in Istan-

TABLE 3. Age-adjusted plasma lipid levels of men versus women in various regions[a]

	Total Cholesterol		LDL Cholesterol		HDL Cholesterol		Cholesterol/HDL-C		Triglycerides	
Location	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Istanbul	202 (0.9)	181 (1.7)	136 (0.8)	117 (1.5)	38 (0.2)	45 (0.4)	5.5 (0.0)	4.2 (0.1)	142 (135)	90 (150)
Adana	184 (1.2)	190 (2.5)	121 (1.1)	129 (2.2)	34 (0.2)	39 (0.6)	5.6 (0.1)	5.1 (0.1)	145 (93)	109 (56)
Trabzon	174 (1.5)	175 (1.3)	115 (1.3)	115 (1.2)	34 (0.3)	42 (0.3)	5.3 (0.1)	4.3 (0.0)	129 (98)	95 (62)
Kayseri	171 (1.5)	179 (1.6)	111 (1.3)	119 (1.4)	34 (0.3)	37 (0.4)	5.1 (0.1)	5.0 (0.1)	128 (84)	121 (82)
Aydin	173 (1.6)	166 (1.8)	107 (1.4)	103 (1.6)	37 (0.3)	43 (0.4)	4.8 (0.1)	4.0 (0.1)	143 (109)	95 (52)
Ayvalik	160 (1.7)	162 (1.6)	100 (1.5)	99 (1.4)	38 (0.3)	42 (0.4)	4.3 (0.1)	3.9 (0.1)	124 (94)	112 (72)
Other[b]	203 (3.7)	194 (10.0)	138 (3.3)	130 (9.2)	37 (0.6)	37 (2.3)	5.7 (0.1)	5.6 (0.3)	155 (123)	165 (235)
n	6085	2932	5943	2908	6085	2932	6085	2932	6085	2932
P (Istanbul vs. Adana)	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	0.43	<0.001	0.53	0.07
P (Adana vs. Trabzon)	<0.001	<0.001	<0.001	<0.001	0.50	<0.001	<0.001	<0.001	<0.001	0.004
P (Trabzon vs. Kayseri)	0.17	0.09	0.03	0.05	0.32	<0.001	0.08	<0.001	0.84	<0.001
P (Kayseri vs. Aydin)	0.40	<0.001	0.03	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	<0.001
P (Aydin vs. Ayvalik)	<0.001	0.08	<0.001	0.04	0.29	0.35	<0.001	0.27	0.001	<0.001
P[c]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a]Mean mg/dl (SEM), except for cholesterol/HDL-C, which is presented as a ratio, and triglycerides, which are presented as an unadjusted mean (SD).
[b]Includes 122 men and 13 women who were not from the region being surveyed but who asked to participate in the study.
[c]*P* = highest value versus lowest value in each category excluding "other."

TABLE 4. Percent distribution of plasma lipids in various regions

	All		Istanbul		Adana		Trabzon		Kayseri		Aydin		Ayvalik		Other	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Plasma Cholesterol																
<200 mg/dl	68	78	52	80	74	71	75	74	79	72	84	90	79	81	48	69
200-219 mg/dl	13	10	16	9	12	9	11	12	9	13	8	4	10	8	12	8
220-239 mg/dl	9	6	12	5	7	11	9	9	7	8	4	3	7	5	16	0
≥240 mg/dl	10	6	20	6	7	9	5	5	5	7	4	3	4	6	24	23
n	6085	2932	2147	523	1089	207	731	748	721	506	674	400	601	535	122	13
LDL-C																
<130 mg/dl	63	72	47	77	66	62	67	65	73	63	82	87	76	78	48	69
130-159 mg/dl	22	19	27	14	22	21	21	26	19	25	13	9	17	15	21	15
≥160 mg/dl	15	9	26	9	12	17	12	9	8	12	5	4	7	7	31	16
n	5943	2908	2082	517	1067	207	721	746	712	497	656	400	590	529	115	12
HDL-C																
≤35 mg/dl	53	26	43	16	65	33	65	23	64	46	45	18	44	23	46	46
36-44 mg/dl	35	40	38	34	29	44	30	40	31	41	43	45	38	41	37	39
≥45 mg/dl	12	34	19	50	6	23	5	37	5	13	12	37	18	36	17	15
n	6085	2932	2147	523	1089	207	731	748	721	506	674	400	601	535	122	13
Cholesterol/HDL-C																
≤3.5	11	30	10	46	8	12	7	24	10	12	16	44	22	37	11	15
3.6-4.5	25	32	22	30	23	32	23	36	28	26	35	33	32	33	15	23
>4.5	64	38	68	24	69	56	70	40	62	62	49	23	46	30	74	62
n	6085	2932	2147	523	1089	207	731	748	721	506	674	400	601	535	122	13
Triglycerides																
<200 mg/dl	85	94	84	96	81	94	87	95	87	91	84	94	87	93	83	85
200-399 mg/dl	13	5	13	3	17	6	12	5	12	7	13	6	11	6	11	8
≥400 mg/dl	2	1	3	1	2	0	1	1	1	2	3	0	2	1	6	7
n	6085	2932	2147	523	1089	207	731	748	721	506	674	400	601	535	122	13

bul had LDL-C levels ≥130 mg/dl. Furthermore, plasma LDL-C levels ≥160 mg/dl occurred most often in men from Istanbul (26%). By comparison, about 17% of U.S. men (20–74 years of age) had LDL-C levels of ≥160 mg/dl (39). The lowest LDL-C levels documented in the Turkish Heart Study were found in men and women from Aydin and Ayvalik.

The LDL-C levels increased with age in parallel with plasma cholesterol levels in both men and women when the means for all populations were compared (Fig. 2B). A very sharp increase in LDL-C level occurred with age from <95 mg/dl at age 20–24 in both men and women to a mean of ~135 mg/dl in men and ~125 mg/dl in women within 30 years. As with plasma cholesterol levels, LDL-C levels in women approached or exceeded those of men after the age of 50 years.

Plasma HDL-C levels. Approximately 50% of Turkish men had HDL-C levels of ≤35 mg/dl (88% had HDL-C levels ≤44 mg/dl) (Table 4). These HDL-C levels are profoundly low compared to other published data. For example, only 16% of German men in the Prospective Cardiovascular Münster (PROCAM) Study had HDL-C levels of <35 mg/dl (40). About one-quarter of Turkish women had HDL-C levels ≤35 mg/dl, and 66% had HDL-C levels ≤44 mg/dl (comparing the means of all regions combined) (Table 4). Istanbul women tended to have the highest HDL-C levels (50% had values ≥45 mg/dl).

Age had no significant effect on HDL-C in either men or women (Fig. 2C). It should be noted that Turkish men had HDL-C levels about 10 mg/dl lower than U.S. men and Turkish women had HDL-C levels about 10–15 mg/dl lower than U.S. women (39).

Cholesterol/HDL-C ratios. The total plasma cholesterol/HDL-C ratio demonstrates the impact of low HDL-C levels even in populations with relatively low plasma cholesterol levels. Approximately 62–70% of the men in Istanbul, Adana, Trabzon, and Kayseri had total cholesterol/HDL-C ratios >4.5, whereas only 49% and 46% of the men in Aydin and Ayvalik, respectively, had this high ratio (Table 4). A ratio >4.5 occurred in 40–62% of the women in Adana, Trabzon, and Kayseri, and in only 23–30% of women in Aydin, Ayvalik, and Istanbul. The women in Istanbul had the lowest total cholesterol/HDL-C ratios (46% had values of 3.5 or less).

Triglyceride levels. Approximately 85% of men and 95% of women had triglyceride levels <200 mg/dl. Hypertri-

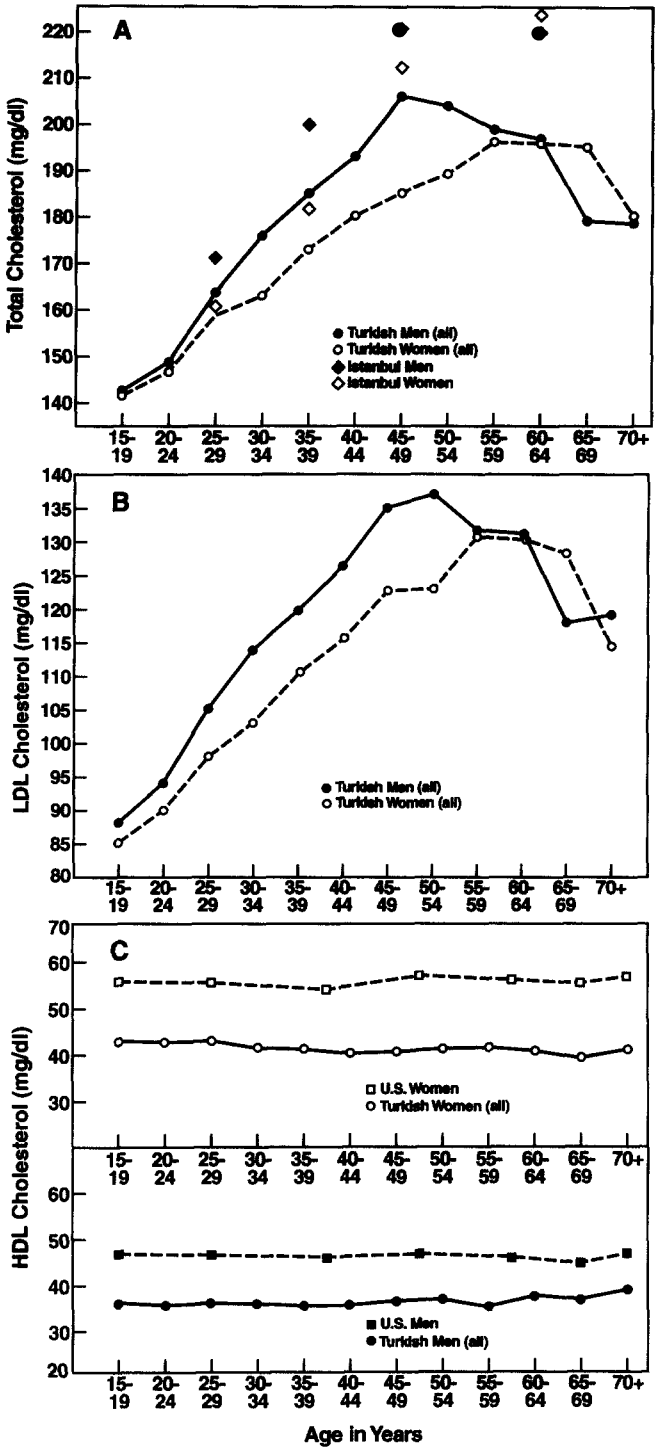

Fig. 2. A. The impact of age on total plasma cholesterol levels is plotted for Turkish men and women from all regions surveyed. Levels for Turkish men and women from Istanbul are shown for comparison. B. The LDL-C levels versus age are shown for men and women from all the survey regions combined. C. Upper panel: the HDL-C levels versus age for Turkish women from all the survey regions are compared to those of U.S. women. Lower panel: the HDL-C levels versus age for Turkish men from all the survey regions are compared to those of U.S. men (NHANES III study) (39). In these graphs the values for 15–19-year-old men and women were included; however, values from this age group were excluded from other analyses because of the small group size (men, n = 32; women, n = 64).

glyceridemia (defined as levels ≥400 mg/dl) is rare in the population surveyed (Table 4). Similar results were obtained by the study of Onat et al. (20).

Factors in the Turkish population influencing plasma lipid levels

Economic status. The higher the salaries in both Turkish men and women, the higher the lipid levels, including plasma cholesterol and LDL-C (**Table 5,** data from all regions combined). This trend was also true for triglyceride levels in men but not in women. Note the striking 44 mg/dl increase in cholesterol levels in men from the lowest salary group compared to those in the highest salary group and the 28 mg/dl increase in women (group I versus group IV). There was no significant change in the HDL-C levels for either men or women with respect to salary.

The effect of salary may be mediated, at least in part, by the diet associated with affluence. When the plasma cholesteryl ester fatty acid results for the lower salary groups (I, II, and III) and the affluent group (IV) were analyzed, a significant increase in saturated fatty acids (16.7% versus 19.7%, $P = 0.032$) and a decrease in polyunsaturated fatty acids (60.8% versus 55.9%, $P = 0.012$) were observed in the affluent group.

Education. In men, higher education levels were associated with higher plasma levels of cholesterol, LDL-C, and triglycerides (Table 5, data from all regions combined). In women, higher education was associated with increased levels of total cholesterol, LDL-C, and HDL-C, and lower triglycerides.

Smoking. The number of cigarettes reported to be smoked per day correlated significantly in men with increased plasma cholesterol, LDL-C, and triglyceride levels comparing individuals who do not smoke with those who smoke more than 20 cigarettes per day (**Table 6**). In the men who were heavy smokers significantly lower levels of HDL-C were observed. Smoking had no obvious effect on the lipids (except for a lowering of HDL-C) of the women who reported smoking more than 20 cigarettes per day; however, very few women were in this category. On the other hand, a larger number of women reported smoking 10–20 cigarettes per day, and when their plasma lipid values were compared to those of nonsmokers, significantly higher cholesterol and LDL-C levels and lower HDL-C levels were observed in the women who smoked (Table 6).

Alcohol consumption. As summarized in Table 6, alcohol consumption was associated with elevated plasma lipid levels in both men and women. However, the increase in HDL-C in women who drank was not significant.

Type of work. As summarized in **Table 7,** participants were divided, where possible, into three categories of work, reflecting physical activity. The plasma cholesterol, LDL-C, and triglyceride levels were lower in both men

TABLE 5. Age- and region-adjusted lipid levels by salary and education in all regions[a]

Variable	Cholesterol		LDL Cholesterol		HDL Cholesterol		Triglycerides		n	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Salary										
I (lowest)	163 (1.2)	169 (1.8)	104 (1.1)	108 (1.7)	36 (0.2)	41 (0.4)	120 (82)	107 (69)	1640	1261
II	179 (1.1)	179 (1.8)	116 (0.9)	115 (1.6)	35 (0.2)	41 (0.4)	138 (108)	102 (117)	2059	1007
III	190 (1.5)	183 (2.3)	125 (1.4)	121 (2.1)	36 (0.3)	40 (0.5)	144 (120)	96 (84)	759	386
IV (highest)	207 (1.3)	197 (2.7)	140 (1.2)	134 (2.4)	37 (0.2)	42 (0.6)	154 (134)	99 (64)	1562	258
P^b	<0.001	<0.001	<0.001	<0.001	0.55	0.37	<0.001	0.15		
Education										
None	155 (2.8)	169 (2.4)	100 (2.4)	108 (2.2)	36 (0.5)	40 (0.6)	112 (69)	106 (67)	264	954
Primary	172 (0.9)	176 (1.9)	110 (0.8)	113 (1.7)	36 (0.2)	40 (0.5)	135 (95)	107 (127)	2333	882
Middle	178 (1.9)	188 (3.4)	115 (1.7)	125 (3.0)	36 (0.3)	42 (0.8)	135 (113)	92 (48)	460	136
High school	189 (1.4)	185 (2.2)	124 (1.2)	123 (2.0)	36 (0.2)	41 (0.5)	141 (140)	93 (68)	968	448
University	198 (1.3)	188 (3.0)	133 (1.1)	127 (2.6)	37 (0.3)	42 (0.7)	147 (121)	84 (55)	1478	214
Postgraduate	199 (2.2)	191 (6.7)	133 (1.9)	128 (5.9)	37 (0.4)	44 (1.6)	147 (119)	81 (36)	400	30
P^c	<0.001	0.002	<0.001	0.001	0.30	0.04	<0.001	0.03		

[a]Mean mg/dl (SEM), except for triglycerides which are presented as an unadjusted mean (SD).
[b]Significance for salary determined between I (lowest) and IV (highest) categories.
[c]Significance for education determined between lowest and highest categories.

and women describing their work as physical labor compared to those in administrative/management-level positions.

Rural versus urban living. In three of the regions surveyed the participants could be divided into those living and working in rural, agricultural communities and those living and working in urban, city environments. As shown in **Table 8,** the total plasma cholesterol and LDL-C levels in the rural populations surveyed tended to be ~20-30 mg/dl lower than those seen in the corresponding urban populations of those regions. In fact, the total cholesterol and LDL-C levels in the Ayvalik rural population (~150 mg/dl and ~90 mg/dl, respectively) are as low as those seen in any population of the world, including the rural Chinese (1). The HDL-C levels in the urban population tended to remain unchanged or even lower compared to those in the rural population, whereas the triglyceride levels tended to be elevated in the urban participants surveyed. Numerous factors may contribute to the differences in the plasma lipid levels in these survey populations, and these analyses will be the subject of future studies.

Lipoprotein[a] levels in the Turkish population

The Lp[a] levels were determined in a total of 800 participants from the six regions. As shown in **Table 9,** the means ± SD were not significantly different among the various populations and ranged from ~11 to 15 mg/dl. When the values for men (n = 526) and women (n = 274) were compared, they were 11.9 ± 13.7 mg/dl (range: 0-76 mg/dl) and 13.7 ± 14.8 mg/dl (range: 0-86 mg/dl), respec-

TABLE 6. Age- and region-adjusted plasma lipid levels by smoking and amount of alcohol consumption in all regions[a]

Variable	Cholesterol		LDL Cholesterol		HDL Cholesterol		Triglycerides		n	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Number of cigarettes										
0/day	180 (1.0)	177 (1.6)	118 (0.8)	114 (1.5)	37 (0.2)	41 (0.4)	135 (110)	104 (94)	2574	2342
1-9/day	179 (1.6)	181 (2.5)	117 (1.4)	119 (2.2)	37 (0.3)	40 (0.6)	129 (84)	97 (77)	673	313
10-20/day	180 (1.0)	184 (2.7)	118 (0.9)	122 (2.4)	35 (0.2)	39 (0.6)	139 (116)	102 (63)	2361	257
20 + /day	190 (1.9)	182 (8.2)	124 (1.7)	122 (7.2)	35 (0.3)	37 (1.9)	164 (127)	109 (54)	477	20
P (0 vs. 10-20/day)	–	0.002	–	<0.001	<0.001	0.003	0.19	0.8		
P (0 vs. 20 + /day)	<0.001	0.50	<0.001	0.28	<0.001	0.02	<0.001	0.8		
Alcohol consumption										
None/little	176 (0.8)	177 (1.6)	115 (0.7)	115 (1.4)	36 (0.1)	41 (0.4)	131 (92)	102 (89)	4300	2790
1-5 glasses/week	193 (1.3)	195 (4.5)	127 (1.2)	130 (4.0)	37 (0.2)	42 (1.0)	150 (151)	102 (68)	1169	72
>5 glasses/week	199 (1.8)	204 (7.6)	131 (1.6)	138 (7.0)	38 (0.3)	42 (1.7)	164 (145)	139 (101)	560	24
P^b	<0.001	<0.001	<0.001	<0.001	<0.001	0.64	<0.001	0.04		

[a]Mean mg/dl (SEM), except for triglycerides which are presented as an unadjusted mean (SD).
[b]Significance for alcohol consumption determined between None/little and >5 glasses per week categories.

TABLE 7. Age- and region-adjusted plasma lipid levels by type of work in all regions[a]

Type of Work	Cholesterol		LDL Cholesterol		HDL Cholesterol		Triglycerides		n	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Physical labor	171 (0.9)	169 (2.0)	110 (0.7)	108 (1.8)	36 (0.2)	41 (0.5)	129 (90)	100 (69)	3159	1914
Office work	192 (1.3)	181 (2.6)	128 (1.1)	119 (2.3)	36 (0.2)	43 (0.7)	142 (110)	81 (42)	1145	359
Administration/manager	203 (1.4)	204 (5.3)	136 (1.3)	141 (4.9)	36 (0.3)	37 (1.3)	164 (159)	136 (109)	1182	43
P	<0.001	<0.001	<0.001	<0.001	–	<0.001	<0.001	<0.001		

[a]Mean mg/dl (SEM), except for triglycerides which are presented as an unadjusted mean (SD).

tively. This difference was not significant at the $P \leq 0.05$ level. Furthermore, there were no significant differences in the mean Lp[a] levels of men versus women in each of the six regions. The characteristics of the population analyzed for Lp[a] values are shown in Table 9.

The distribution of Lp[a] levels was shifted toward lower values, with 25% of the population having levels ≤3 mg/dl, including a high percentage with values <1 mg/dl (**Fig. 3**). The value for the 75th percentile was ~19 mg/dl and for the 90th percentile ~30 mg/dl for both men and women.

One of the factors thought to elevate Lp[a] levels in women is menopause (41–44), as Lp[a] levels appear to increase with age. In the Turkish population Lp[a] levels did not change significantly with age in the 274 women studied. The mean value for women less than 50 years of age (n = 182) was 14.2 mg/dl compared to 12.8 mg/dl for women 50 years or older (n = 92). The Lp[a] values for women less than 45 years of age and 45 years or older were 12.9 mg/dl and 14.9 mg/dl, respectively (not significantly different). The use of estrogen replacement therapy or birth control pills is rare in Turkish women, especially those living outside of Istanbul. It is unlikely that these therapies had an effect on the values obtained.

No significant correlation was observed between Lp[a] levels and plasma cholesterol, LDL-C, or HDL-C levels for men and women. A significant inverse association was observed, however, between Lp[a] levels and triglyceride levels. The mean Lp[a] value was 8.5 ± 10.9 mg/dl in participants with triglyceride levels ≥200 mg/dl (n = 89),

TABLE 8. Comparison of age-adjusted mean plasma lipid levels for rural and urban populations surveyed[a]

Lipids	Trabzon Region[b]			Kayseri Region[c]			Ayvalik Region[d]		
	Rural	Urban	*P*	Rural	Urban	*P*	Rural	Urban	*P*
Total Cholesterol									
Males	173 (1.5) n = 505	200 (2.6) n = 169	<0.001	159 (2.8) n = 201	189 (2.9) n = 141	<0.001	149 (1.9) n = 304	188 (2.3) n = 212	<0.001
Females	178 (1.4) n = 619	201 (4.9) n = 50	<0.001	180 (1.9) n = 376	190 (4.0) n = 90	0.03	150 (1.9) n = 340	194 (3.0) n = 141	<0.001
LDL Cholesterol									
Males	115 (1.3) n = 499	137 (2.3) n = 166	<0.001	103 (2.5) n = 199	125 (2.6) n = 140	<0.001	90 (1.7) n = 297	124 (2.0) n = 209	<0.001
Females	118 (1.2) n = 618	134 (4.4) n = 49	<0.001	120 (1.7) n = 367	127 (3.5) n = 90	0.07	93 (1.7) n = 335	129 (2.6) n = 141	<0.001
HDL Cholesterol									
Males	35 (0.3) n = 505	33 (0.4) n = 169	<0.001	34 (0.5) n = 201	33 (0.5) n = 141	<0.001	38 (0.4) n = 304	37 (0.5) n = 212	0.43
Females	43 (1.1) n = 619	39 (0.3) n = 50	<0.001	37 (0.4) n = 376	37 (0.7) n = 90	0.07	42 (0.4) n = 340	42 (0.7) n = 141	0.80
Triglycerides									
Males	123 (100) n = 505	152 (87) n = 169	<0.001	122 (76) n = 201	155 (103) n = 141	<0.001	112 (84) n = 304	135 (95) n = 212	0.004
Females	91 (51) n = 619	142 (139) n = 50	<0.001	121 (86) n = 376	121 (69) n = 90	–	108 (76) n = 340	111 (58) n = 141	0.7

[a]Mean mg/dl (SEM), except for triglycerides, which are presented as an unadjusted mean (SD).

[b]The rural population of the Trabzon region that was surveyed included Tonya (a small town known for cheese production) and several small villages. The population was primarily involved in agricultural work. The urban population, primarily business people and professionals, lived and worked in Vakfikebir.

[c]The rural population included the inhabitants of several villages primarily involved in agricultural work. The urban population included business people, professionals, and their families. Members of the Rotary and Lions Clubs participated.

[d]Inhabitants of several small villages involved primarily in agriculture (olive production) were surveyed. The urban population lived and worked in Ayvalik. Rotary Club members and their families participated.

TABLE 9. Mean lipoprotein[a] values (mg/dl) by location

Location	n	Mean (SD)
Istanbul	158	12.0 (15.2)
Adana	101	14.7 (16.3)
Trabzon	263	11.3 (12.8)
Kayseri	89	12.6 (13.5)
Aydin	94	13.0 (11.7)
Ayvalik	95	14.1 (15.9)

Population characteristics (all locations combined)

Subjects	Number	Age[a]	Body Mass Index[a]	Plasma Cholesterol[a]
		yr	*kg/m²*	*mg/dl*
Males	526	41 ± 14[b]	25.6 ± 3.7[b]	185 ± 41[b]
Females	274	42 ± 15	26.3 ± 4.7	180 ± 44

[a]Mean ± SD.
[b]Values for males and females are not significantly different.

TABLE 10. Apolipoprotein E phenotype and allele frequencies (% in various populations[a]

	United States	Germany	Finland	Sudan	Japan	Turkey (n = 836
Phenotype						
E4/4	3.0	2.8	5.9	8.7	1.3	1.1
E4/3	14.0	22.9	35.5	35.9	11.3	12.9
E3/3	58.0	59.8	46.8	39.8	72.1	74.2
E3/2	22.0	12.0	9.9	9.7	13.8	10.6
E2/2	1.3	1.0	0.5	1.0	0.6	0.4
E4/2	2.0	1.5	1.5	4.9	0.9	0.8
Allele						
ε4	11.0	15.0	24.4	29.1	7.4	7.9
ε3	76.0	77.3	69.5	61.9	84.6	86.0
ε2	13.0	7.7	6.2	8.1	8.0	6.1
Reference	45	46	47	47	47	

[a]Modified from Mahley, R. W., and S. C. Rall, Jr. 1994. *In* T Metabolic and Molecular Bases of Inherited Disease, Seventh Editio C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGra Hill, New York. Reproduced with the permission of McGraw-Hill, In

whereas the mean level was 13.1 ± 14.4 mg/dl in participants with triglyceride levels <200 mg/dl (n = 711) (P = 0.004).

Apolipoprotein E isoforms in the Turkish population

Apolipoprotein E phenotype and allelic frequency. The apoE phenotype was determined in 8,366 participants of the Turkish Heart Study. Interestingly, the phenotype and allelic distributions were different in the Turkish population from those seen in many other population groups reported. The results of the present study are compared to others in **Table 10** (see ref. 22 for a more complete comparison). The ε3 allele was most common, with a frequency of 86% (74.2% of the participants had an apoE3/3 phenotype). This frequency is higher than those seen in other populations. In the Turkish population, the frequencies of the ε4 and ε2 alleles were 7.9% and 6.1% respectively, which are lower than in most other populations. The Turkish values were more similar to those o the Japanese than the U.S. or European populations (fo review, see ref. 22).

Impact of apoE phenotype on lipid levels. As summarized i **Table 11** (combining all regions), the interaction betwee the ε2 versus the ε4 allele on plasma lipids was analyze by comparing the lipid levels of participants wit apoE2/2, apoE3/2, apoE3/3, apoE4/3, and apoE4/ phenotypes. The apoE3/2 phenotype was associated wit lower plasma cholesterol and LDL-C levels in both me and women compared to results obtained in apoE3/3 in dividuals. In men with the apoE3/2 phenotype, th plasma cholesterol and LDL-C levels were lower b

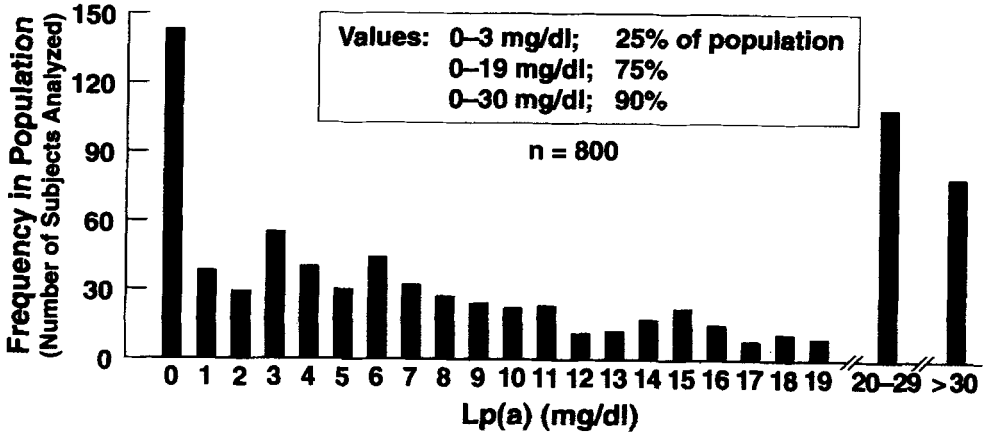

Fig. 3. Frequency distribution of Lp[a] levels in the Turkish population: 0 mg/dl = values that ranged from 0 mg/dl to 0.9 mg/dl; 1 mg/dl = values that ranged from 1.0 mg/dl to 1.9 mg/dl; etc. Approximately 25%, 75%, and 90% of the population had Lp[a] levels of ≤3 mg/dl, ≤19 mg/dl, and ≤30 mg/dl, respectively.

TABLE 11. Mean plasma lipid values (mg/dl ± SD) versus apoE phenotype

	ApoE2/2					ApoE4/4	
	All	Excluding[a]	ApoE3/2	ApoE3/3	ApoE4/3	All	Excluding[b]
Males							
Cholesterol	192 ± 68	161 ± 32	175 ± 47	186 ± 44	190 ± 43	188 ± 46	181 ± 41
	n = 23	n = 17	n = 586	n = 4290	n = 780	n = 81	n = 67
LDL-C	102 ± 32	93 ± 25	109 ± 39	123 ± 38	127 ± 38	123 ± 40	122 ± 38
	n = 19	n = 16	n = 563	n = 4209	n = 755	n = 78	n = 67
HDL-C	34 ± 7	35 ± 7	37 ± 8	36 ± 7	36 ± 7	37 ± 8	37 ± 8
	n = 23	n = 17	n = 586	n = 4290	n = 780	n = 81	n = 67
Triglycerides	252 ± 236	170 ± 116	147 ± 117	136 ± 111	147 ± 113	142 ± 93	109 ± 43
	n = 23	n = 17	n = 586	n = 4290	n = 780	n = 81	n = 67
Probability	E2/2 vs. E3/3	E2/2 vs. E3/3	E3/2 vs. E3/3	E3/2 vs. E4/3	E4/3 vs. E3/3	E4/4 vs. E3/3	E4/4 vs. E3/3
Cholesterol	NS	[d]	[c]	[c]	[d]	NS	NS
LDL-C	[d]	[c]	[c]	[c]	[d]	NS	NS
HDL-C	NS	NS	NS	NS	NS	NS	NS
Triglycerides	[c]	NS	[d]	NS	[d]	NS	[d]
Females							
Cholesterol	160 ± 71	125 ± 15	160 ± 40	176 ± 38	179 ± 46	196 ± 43	191 ± 38
	n = 13	n = 10	n = 308	n = 2011	n = 323	n = 17	n = 16
LDL-C	72 ± 27	64 ± 10	96 ± 33	115 ± 35	115 ± 35	131 ± 39	128 ± 36
	n = 11	n = 10	n = 305	n = 1999	n = 320	n = 17	n = 16
HDL-C	41 ± 11	44 ± 10	43 ± 9	42 ± 9	42 ± 9	44 ± 11	44 ± 11
	n = 13	n = 10	n = 308	n = 2011	n = 323	n = 17	n = 16
Triglycerides	189 ± 253	83 ± 19	102 ± 68	99 ± 66	113 ± 184	103 ± 63	93 ± 45
	n = 13	n = 10	n = 308	n = 2011	n = 323	n = 17	n = 16
Probability	E2/2 vs. E3/3	E2/2 vs. E3/3	E3/2 vs. E3/3	E3/2 vs. E4/3	E4/3 vs. E3/3	E4/4 vs. E3/3	E4/4 vs. E3/3
Cholesterol	[d]	[c]	[c]	[c]	NS	[d]	NS
LDL-C	[c]	[c]	[c]	[c]	NS	[d]	NS
HDL-C	NS	NS	NS	NS	NS	NS	NS
Triglycerides	NS	NS	NS	NS	[d]	NS	NS

[a]ApoE2/2 individuals excluding those with cholesterol levels ≥220 mg/dl.
[b]ApoE4/4 individuals excluding those with triglyceride levels ≥200 mg/dl.
[c]$P < 0.001$; [d]$P \leq 0.05$ to 0.001; NS, not significant ($P > 0.05$).

11 mg/dl and 14 mg/dl, respectively, compared to those with the apoE3/3 phenotype. In the apoE3/2 women, the plasma cholesterol and LDL-C levels were 16 mg/dl and 19 mg/dl lower, respectively. The apoE2/2 phenotype was prominently associated with hypolipidemia, except in those individuals displaying type III hyperlipoproteinemia. As shown in Table 11, when the hyperlipidemic apoE2 homozygotes (arbitrarily defined as having cholesterol levels ≥220 mg/dl) were excluded from the comparisons (6 men out of 23 and 3 women out of 13), a major difference was seen in the total cholesterol and LDL-C levels compared to those with the apoE3/3 phenotype. The plasma cholesterol and LDL-C levels were lower by 25 mg/dl and 30 mg/dl, respectively, in men, and both levels were 51 mg/dl lower in women.

The occurrence of ε4 in the individuals with apoE4/3 was associated with a very slight increase in plasma cholesterol and LDL-C in men, but not in women, as compared to individuals with apoE3/3. However, an elevation in the total plasma cholesterol and LDL-C levels (17 mg/dl and 16 mg/dl, respectively) was seen in apoE4 homozygous women but not in apoE4 homozygous men. As shown in Table 11, no significant differences were observed in the HDL-C levels among either men or women with the various apoE phenotypes. The mean levels of HDL-C were 34–37 mg/dl in men and 41–44 mg/dl in women among the groups.

Men with the apoE3/2 or apoE4/3 phenotype displayed an increase in the triglyceride levels of 11 mg/dl compared to apoE3 homozygous men. Likewise, a marked elevation was seen in the triglycerides of apoE2 homozygous subjects compared to those with the apoE3/3 phenotype. However, when the hyperlipidemic apoE2 homozygous individuals were excluded, the elevation of triglyceride levels disappeared (Table 11). Apolipoprotein E4 homozygosity was not associated with a difference in triglyceride levels unless those with hypertriglyceridemia (arbitrarily defined as levels ≥200 mg/dl) were excluded. Then the apoE4/4 phenotype was associated with lower triglyceride levels compared to the apoE3/3 phenotype in men (Table 11). In women no significant association of the ε2 or ε4 allele was observed with plasma triglyceride levels except for a 14 mg/dl increase associated with the apoE4/3 phenotype as compared to apoE3 homozygous women.

Percent allelic frequency versus plasma cholesterol and LDL-C levels. The ε2 allele is clearly overrepresented in individuals with the lowest plasma cholesterol and LDL-C levels (≤149 mg/dl and ≤99 mg/dl, respectively), and un-

derrepresented in those with the highest levels (**Table 12**). On the other hand, the ε4 allele appears to be overrepresented in the group with the highest plasma cholesterol and LDL-C levels. These observations are consistent with those described in Table 11 (i.e., the ε2 allele is associated with lower cholesterol and LDL-C levels and the ε4 allele is associated with a tendency for higher levels).

Percent allelic frequency versus HDL-C levels. No clear tendency was seen for an overrepresentation or underrepresentation of the ε2 or ε4 allele with either a low or high HDL-C, except for the possible overrepresentation of ε4 with low HDL-C in males (Table 12).

Percent allelic frequency versus triglyceride levels. Both the ε2 and ε4 alleles appear to be overrepresented in men and women with the highest plasma triglyceride levels (≥200 mg/dl) (Table 12).

Screening for the 3500 mutation of apolipoprotein B-100

The mutation involving a single base change at residue 3500 of apoB-100 (glutamine substituted for arginine) which is characteristic of familial defective apoB-100, was not detected in the survey of 2,450 participants in the Turkish Heart Study. As summarized in **Table 13,** this survey includes 1,063 subjects with cholesterol levels ≥220 mg/dl and an additional 1,387 individuals. Therefore, by comparison to the U.S. and German populations (for review, see ref. 25), this mutation is extremely rare in Turkey.

DISCUSSION

The present study assesses the magnitude of CAD risk in the population of Turkey as ascertained by measurement of plasma lipid levels in six regions of Turkey that reflect the dietary diversity of this country. These data confirm and extend prior results of plasma cholesterol and triglyceride levels in the Turkish population (20). Fatty acid analyses of plasma cholesteryl esters (Table 2) demonstrate the differences in diet. The diets in Adana, Trabzon, and Kayseri, noted for their high meat and dairy product content, appear to be relatively high in saturated fats, as reflected by the levels of C16:0 (palmitate) and C18:0 (stearate) cholesteryl ester fatty acids. The plasma cholesteryl ester fatty acids in subjects from Aydin are enriched in C18:2 (linoleate), reflecting a diet high in polyunsaturated fats and vegetable oils. Likewise, the high level of C18:1 (oleate) cholesteryl ester fatty acids in the plasma samples from Ayvalik confirms the widespread consumption of monounsaturated fats (olive oil) in this population. The most diverse diet was expected in the

TABLE 12. Apolipoprotein E allelic frequencies (%) versus plasma lipid levels

	n		ε2		ε3		ε4	
	Male	Female	Male	Female	Male	Female	Male	Female
Plasma Cholesterol								
≤149 mg/dl	1215	738	8.8[a]	11.2[a]	84.3	83.1	6.9[a]	5.7[b]
150–199 mg/dl	2606	1346	5.2	5.3	86.3	87.1	8.5	7.6
≥200 mg/dl	1860	603	4.6[a]	4.3[a]	86.1	88.0	9.3[a]	7.7[b]
LDL-C								
≤99 mg/dl	1676	1042	8.4[a]	10.4[a]	84.1	83.0	7.5[a]	6.6[a]
100–129 mg/dl	1763	882	5.3	4.5	86.5	88.1	8.1	7.4
130–159 mg/dl	1213	492	3.9	4.6	87.3	88.6	8.8	6.8
≥160 mg/dl	895	252	3.6[a]	2.6[a]	86.9	88.5	9.5[a]	8.9[a]
HDL-C								
≤34 mg/dl	2586	512	5.6	6.4	85.1	85.5	9.3[a]	8.1
35–44 mg/dl	2359	1210	5.9	6.5	86.0	86.2	8.1	7.3
45–54 mg/dl	613	721	6.5	7.1	86.6	87.1	6.9	5.8
≥55 mg/dl	123	244	5.3	7.0	88.7	85.0	6.0[a]	8.0
Triglycerides								
≤49 mg/dl	303	336	4.1[a]	6.9[a]	88.9	85.1	7.0[a]	8.0
50–99 mg/dl	2204	1373	5.3	6.4	86.9	87.1	7.8	6.5[c]
100–149 mg/dl	1441	583	6.1	6.8	85.5	86.5	8.4	6.7
150–199 mg/dl	839	235	5.7	5.8	85.5	86.1	8.8	8.1
≥200 mg/dl	894	160	7.3[a]	10.0[a]	82.6	79.0	10.1[a]	10.0[c]

[a]Values for the ε2 and ε4 allelic frequencies, comparing the results for individuals with the highest versus the lowest lipid values, are significantly different ($P < 0.001$).

[b]Values for the ε4 allelic frequency, comparing the results for women with the highest versus the lowest plasma cholesterol levels, are significantly different ($P = 0.003$).

[c]Values for the ε4 allelic frequency, comparing the triglyceride results for women in the 50–99 mg/dl group with those in the ≥200 mg/dl group, are significantly different ($P = 0.003$).

TABLE 13. Populations surveyed for familial defective apolipoprotein B-100 (3500 mutation)

Location	Number of Individuals Screened	
	Group I[a]	Group II[b]
Istanbul	313	603
Adana	193	
Trabzon	217	250
Kayseri	154	350
Aydin	78	
Ayvalik	48	171
Other[c]	60	13
Total	1063	1387

[a]Group I included individuals with plasma cholesterol levels ≥220 mg/dl.
[b]Group II included individuals with plasma cholesterol levels <220 mg/dl.
[c]Individuals referred to the Lipid Clinic at the American Hospital (Istanbul) but not part of the Turkish Heart Study.

Istanbul population, and as shown in the Results section under *Economic status,* significant dietary variation was seen in this group. In general, the plasma cholesterol and LDL-C levels were higher in the populations consuming the highest levels of saturated fats and lower in those consuming diets rich in polyunsaturated and monounsaturated fats. These results are consistent with a large body of evidence from several studies (2, 48–55). However, the present study considers these dietary effects in an ethnic group within a single country.

The highest plasma cholesterol and LDL-C levels were seen in Istanbul, especially in men. The magnitude of risk from hypercholesterolemia (≥240 mg/dl) and elevated LDL-C (≥160 mg/dl) is greatest in Istanbul men (20–25% have these high-risk levels). Furthermore, almost 50% of the men in Istanbul have total cholesterol levels >200 mg/dl and LDL-C levels >130 mg/dl. In Adana, Trabzon, and Kayseri, ~25–35% of both men and women have plasma cholesterol and LDL-C levels greater than 200 mg/dl and 130 mg/dl, respectively. In Aydin and Ayvalik, ~10–20% of the men and women have levels of cholesterol and LDL-C in excess of 200 mg/dl and 130 mg/dl, respectively. Thus, although the magnitude of the problem may not be as great in Turkey as in some industrialized nations, in certain parts of Turkey a large segment of the population is at risk, especially considering the prevalence of smoking and low HDL-C levels.

The HDL-C levels in Turkish men and women were strikingly low in all regions of the country. In men the mean HDL-C levels ranged from 34 to 38 mg/dl (by comparison, the U.S. mean for men is ~47 mg/dl), and in women the mean HDL-C levels ranged from 37 to 45 mg/dl (the U.S. mean for women is ~56 mg/dl) (34). Even taking into account the relatively low levels of plasma cholesterol and LDL-C, the total cholesterol/HDL-C ratio is high. The mean values for the men of all regions are equal to or greater than 4.5, a ratio of total cholesterol/HDL-C considered by some to represent high risk (11, 36–38), and this ratio is greater than 4.5 for the women of Adana and Kayseri (Table 3).

The results summarized in **Table 14,** which compares lipid values in the U.S., Germany, and Turkey, emphasize several important points. The abnormally low HDL-C in the Turkish population may be, at least in part, genetically determined. Turkish men and women living in Germany (56) have low HDL-C levels similar to those obtained for Turks living in Turkey. The low HDL-C levels in the Turkish population do not appear to relate to diet, as the diets are very different in the various regions of Turkey surveyed; however, the HDL-C levels are uniformly low in all regions. In addition, despite lower cholesterol levels in the Turks, the total cholesterol/HDL-C ratio is higher in Istanbul men and women than in either German or U.S. men and women. The low HDL-C level and the high total plasma cholesterol/HDL-C ratio are predictive of increased CAD risk, as pointed out in

TABLE 14. Comparison of plasma lipid levels (mg/dl) among different populations

Lipids	Americans (in the United States)[a]		Germans (in Germany)[b]		Turks (in Germany)[b]		Turks (in Istanbul)[c]		Turks (in Ayvalik)[d]	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Total cholesterol	206	208	218	211	193	174	202	181	160	162
LDL-C	132	126	144	132	124	108	136	117	100	99
HDL-C	46	55	47	60	38	46	38	45	38	42
Total cholesterol/HDL-C	4.5	3.8	4.6	3.5	5.1	3.8	5.5	4.2	4.3	3.9
Triglycerides	149	129	119	88	135	88	142	90	124	112

[a]Caucasian men and women (age 20–74 years, 1988–1991); NHANES III study (34).
[b]Prospective Cardiovascular Münster Study (PROCAM) (56). A significant difference ($P < 0.001$) was observed between Turkish and German sex-matched values for total cholesterol, LDL-C, and HDL-C.
[c]Turkish Heart Study (Istanbul survey population).
[d]Turkish Heart Study (Ayvalik survey population).

several studies (1, 11, 36–38). Even in the Ayvalik population with very low cholesterol levels, the total cholesterol/HDL-C ratios for men and women only approach what some believe to be protective (36–38) and are similar to the ratios seen for men and women in the United States and Germany (known to be high-risk populations).

Triglyceride levels in Turkish men living in Turkey are similar to those seen in American and German men and are similar in Turks living in Germany (56). Turkish women have somewhat lower levels than those seen in the men, and these levels are similar to those of Turkish women living in Germany. Several studies have implicated high triglyceride levels as a CAD risk factor, especially in the context of low HDL-C (7, 57).

A comparison of several of the lipoprotein values in combination provides some insights into the potential importance of the various lipid parameters in the Turkish population. As shown in **Table 15,** 36.5% of Turkish men and 27.4% of Turkish women have LDL-C levels of >130 mg/dl. However, about 50% of the men and 25% of the women with LDL-C levels >130 mg/dl also have HDL-C levels of ≤35 mg/dl. Thus, the combination of high LDL-C (>130 mg/dl) and low HDL-C (≤35 mg/dl) occurs in 18.6% of the men and 6.7% of the women in this survey population. In the Istanbul men about 50% have LDL-C levels >130 mg/dl, and the combination of LDL-C level >130 mg/dl and HDL-C level ≤35 mg/dl occurs in 21.7% of the men in the study. In addition, the PROCAM study has highlighted the importance of the LDL-C/HDL-C ratio of ≥5 and a plasma triglyceride ≥200 mg/dl as an important triad predicting coronary heart disease (57). Likewise, the Helsinki Heart Study has drawn attention to the importance of this triad as a risk factor (58). As shown in Table 15, 10.7% of Turkish men and 3.5% of Turkish women have the very high LDL-C/HDL-C ratio of ≥5. About 2.6% of men and 0.7% of women have the combination of LDL-C/HDL-C ratio ≥5 and a triglyceride level ≥200 mg/dl. Approximately 3.7% of Istanbul men have this same combination (high LDL-C/HDL-C ratio and high triglyceride level). In the PROCAM study, 4.3% of the men had this combination of risk factors; however, this subgroup had the highest incidence of cardiac events and accounted for nearly 25% of all the infarctions observed (57).

One of the most dramatic predictors of high cholesterol and LDL-C levels in Turkish men and women is salary: the higher the salary, the higher the plasma cholesterol. Comparison of the lowest- with the highest-paid Turks revealed a 44 mg/dl difference in men and a 28 mg/dl difference in women. In the U.S. there is no such profound direct correlation of cholesterol levels with salary (59). In fact, in U.S. men there is an inconsistent change, whereas in U.S. women there is no change or even a decline in total cholesterol at the highest salary levels (59). A similar trend is noted when comparing the level of education in Turkish men and women: higher degrees and more education are associated with higher cholesterol and LDL-C levels. In the U.S. the total cholesterol levels are similar across a range of educational levels (59, 60), and overall in men and women (ages 20–74 years) there is an inverse relationship with education (61). In the United States education has been shown to be the best predictor of good health, and a higher risk of coronary heart disease is associated with lower levels of education (61). This is not likely to be true in Turkey at the present time.

It is reasonable to speculate that the recent rapid economic development of Turkey has resulted in the availability of large quantities of high-fat, high-calorie foods that can be obtained with little energy expenditure by those individuals with the necessary financial resources.

TABLE 15. Percent distribution of combinations of lipid and lipoprotein levels in the Turkish population

A. LDL-C (≤130 mg/dl or >130 mg/dl) versus HDL-C levels (mg/dl)

Turkish Men				Turkish Women			
≤130 mg/dl	>130 mg/dl			≤130 mg/dl	>130 mg/dl		
63.5%	36.5%			72.6%	27.4%		
	HDL-C Level				HDL-C Level		
	≤35	36–44	≥45		≤35	36–44	≥45
	18.6%	13.0%	4.9%		6.7%	12.4%	8.2%

B. LDL-C/HDL-C ratio (<5 or ≥5) versus triglyceride levels

<5	≥5			<5	≥5		
89.3%	10.7%			96.5%	3.5%		
	Triglyceride Level				Triglyceride Level		
	<200 mg/dl	≥200 mg/dl			<200 mg/dl	≥200 mg/dl	
	8.1%	2.6%			2.8%	0.7%	

In fact, our data demonstrate an association between affluence and a significant increase in the level of saturated fatty acids associated with plasma cholesteryl ester fatty acids. Although undoubtedly other factors could be invoked to explain the direct correlation between increased salary and increased cholesterol, the data are consistent with an increased saturated fat consumption correlating with affluence. Currently, little public education is available to advocate heart-healthy lifestyles in Turkey.

Smoking has been clearly established as a modulator of lipid values and as a risk factor for CAD. Cigarette smoking has been shown to elevate plasma cholesterol levels in men and women under the age of 60 years (62) and to decrease HDL-C levels in some studies (for review, see ref. 63). It is a major health problem in Turkey, where 46% to 73% (depending on region) of male participants of the Turkish Heart Study smoke. Among women, the prevalence of smoking is more variable. In the largest cities, Istanbul and Adana, 43% and 32% of the women, respectively, also report smoking. As few as 4% of the women in the Trabzon region, a more conservative area, smoke. By comparison, in 1987, 32% of U.S. men and 27% of U.S. women smoked cigarettes (64). In Turkish men, smoking 20 or more cigarettes per day was associated with a significant increase in total cholesterol, LDL-C, and triglyceride levels, and a decrease in HDL-C levels. In Turkish women who smoked 10–20 cigarettes/day there was an increase in total cholesterol and LDL-C levels and a decrease in HDL-C levels (Table 6). Very few women in the Turkish Heart Study smoked 20 or more cigarettes per day.

Plasma Lp[a] concentration and distribution profile vary among different populations and ethnic groups (65–67). However, Lp[a] levels show little variation throughout life and are not significantly affected by age, sex, diet, or most pharmacological interventions (15, 17). One factor that has been reported to elevate Lp[a] levels is menopause (41–44). Plasma Lp[a] concentrations above 20–30 mg/dl were widely shown in retrospective studies to be a risk factor for CAD (15, 68–70). On the other hand, two prospective studies failed to establish this relationship (18, 19).

In the Turkish population, the mean Lp[a] levels for men and women were ~12–14 mg/dl with values of ~30 mg/dl at the 90th percentile. Menopause did not appear to have an impact on Lp[a] levels in Turkish women. Furthermore, no significant correlation was seen between Lp[a] levels and total cholesterol, LDL-C, or HDL-C levels; however, an inverse correlation was observed between low Lp[a] levels (8.5 mg/dl) and high triglyceride levels (≥200 mg/dl) and conversely between high Lp[a] levels (13.1 mg/dl) and low triglyceride levels (<200 mg/dl). The impact of Lp[a] on coronary heart disease in the Turkish population remains to be determined.

Apolipoprotein E, a M_r = 34,000 protein with three common allelic forms encoded by its gene on chromosome 19, plays a major role in cholesterol homeostasis and in controlling lipoprotein metabolism (21, 22). The three common isoforms are apoE2, apoE3, and apoE4, which differ by a single amino acid at residue 112 or 158 (21, 22). The apoE phenotype and allelic frequencies, as determined in the Turkish Heart Study, were surprising, given the close proximity of Turkey to Europe. However, the similarity between the results obtained in Japan and those obtained in Turkey (Table 10) may reflect the Central Asian origin of the Turkish people. A very similar distribution of apoE phenotypes was observed in the six different regions surveyed (data not shown). These data suggest significant homogeneity among the people in the different regions surveyed in the Turkish Heart Study.

The Turkish Heart Study represents the single largest apoE phenotyping survey performed within a single country by the same laboratory. Sufficient numbers of samples were obtained to compare differences among the apoE alleles in males versus females. Apolipoprotein E polymorphism is one of the common genetic factors accounting for the interindividual variation in cholesterol levels in different populations. The ε2 allele has been strongly associated with lower total cholesterol and LDL-C levels compared to the ε3 allele, whereas the ε4 allele is associated with higher levels (23, 24, 71–74). Data from the Turkish Heart Study clearly demonstrate that the ε2 allele is associated with a decrease in total cholesterol and LDL-C levels, and both men and women with apoE3/2 had total cholesterol and LDL-C levels ranging from 11 to 19 mg/dl less than those observed in individuals homozygous for apoE3 (results summarized in **Table 16**). Likewise, a significant decrease in total cholesterol and LDL-C levels was observed in individuals with the apoE2/2 phenotype, especially when apoE2 homozygous individuals displaying type III hyperlipoproteinemia were excluded. As described in Table 16, it was necessary to exclude 6 of 23 men with type III hyperlipoproteinemia to demonstrate the impact of the ε2 allele on total cholesterol in men only. On the other hand, the ε4 allele in males with the apoE4/3 phenotype was associated with a slight increase in total cholesterol and LDL-C levels (~4 mg/dl each), whereas men with the apoE4/4 phenotype did not have a significant increase compared to males with the apoE3/3 phenotype. Women with the apoE4/3 phenotype had no significant increase in total cholesterol or LDL-C levels compared to women with apoE3/3 but demonstrated a marked increase in these levels (20 mg/dl and 16 mg/dl, respectively) with the apoE4/4 phenotype.

Various studies have failed to demonstrate a consistent effect of the ε alleles on HDL-C levels. Dallongeville, Lussier-Cacan, and Davignon (74) could not demonstrate a clear association with the ε2 allele; however, they found,

TABLE 16. Differences in lipid levels among individuals with apoE2/2 or apoE3/2 and apoE4/4 or apoE4/3 versus apoE3/3[a]

	ApoE2/2		ApoE3/2		ApoE4/3		ApoE4/4	
	Male	Female	Male	Female	Male	Female	Male	Female
Total cholesterol	NS[b]	↓(−16)	↓(−11)	↓(−16)	↑(+4)	NS	NS	↑(+20)
LDL-C	↓(−21)	↓(−43)	↓(−15)	↓(−19)	↑(+4)	NS[c]	NS	↑(+16)
HDL-C	NS	NS	NS	NS	NS[d]	NS	NS	NS
Triglycerides	↑(+116)	NS[e]	↑(+11)	NS[f]	↑(+11)	↑(+14)	NS[g]	NS

[a]Significant increase (↑) or decrease (↓) (± mg/dl) in the lipid levels with apoE2/2 or apoE3/2 and apoE4/4 or apoE4/3 compared to the lipid values of individuals with apoE3/3 (NS = no significant change from apoE3/3; $P > 0.05$).

[b]When the six hyperlipidemic apoE2/2 individuals (cholesterol ≥220 mg/dl) are excluded from the total of 23 apoE2/2 individuals, there is a 25 mg/dl decrease in cholesterol compared to apoE3/3 (Table 11).

[c]There is an overrepresentation of ε4 at the LDL-C ≥160 mg/dl (Table 12).

[d]There appears to be an overrepresentation of ε4 at the HDL-C level of ≤34 mg/dl (Table 12).

[e]Small sample size (13 individuals) and large variance; however, the mean triglyceride level was increased 90 mg/dl (Table 11).

[f]There is an overrepresentation of ε2 at the triglyceride level of ≥200 mg/dl (Table 12).

[g]If apoE4/4 individuals with hypertriglyceridemia (≥200 mg/dl) are excluded, the apoE4/4 individuals appear to have a decrease in triglyceride levels (Table 11).

using pooled data from numerous studies, that the apoE4/3 phenotype was associated with lower HDL-C levels compared to apoE3/3. In the Turkish population, which has very low levels of HDL-C in both men and women, no significant impact on HDL-C levels could be found in apoE2/2, apoE3/2, apoE4/3, or apoE4/4 individuals compared to those with the apoE3/3 phenotype (Table 16). However, it appeared that the ε4 allele was definitely overrepresented in men with HDL-C levels ≤34 mg/dl compared to men with HDL-C levels >45 mg/dl (Table 12). Thus, apoE does not appear to have a major impact on HDL-C levels and does not explain the low levels of HDL-C in this population. Other genetic and environmental factors are under investigation.

The association of the ε allele with triglyceride levels has not been entirely consistent from study to study (24, 72, 75). Utermann et al. (23, 72) have reported that ε2 was frequently associated with hypertriglyceridemia, and Sing and Davignon (75) found that the ε4 allele was associated with lower plasma triglycerides. Using meta-analysis to compare the data in 45 populations in 17 countries, Dallongeville et al. (74) found that the ε2 allele of individuals with apoE2/2 and apoE3/2 was associated with higher triglyceride levels, but also demonstrated higher triglyceride levels in individuals with the apoE4/3 phenotype; on the other hand, however, the apoE4/4 phenotype was not associated with elevated triglyceride levels. Previously, it was demonstrated that ε4 was overrepresented in patients with type V hyperlipidemia (76). In the Turkish Heart Study, men, but not women, with apoE3/2 were found to have triglycerides that were elevated by 11 mg/dl compared to men homozygous for apoE3 (Table 16). However, the ε2 allele appeared to be overrepresented in women with triglyceride levels ≥200 mg/dl compared to women with lower triglyceride levels (Table 12). Homozygosity for apoE2 resulted in a very marked hypertriglyceridemia in both men and women (+116 mg/dl and +90 mg/dl, respectively, compared to apoE3/3 individuals). However, the triglyceride value in apoE2 homozygous women did not reach statistical significance because of the small sample size and the large variance. Interestingly, both men and women with apoE4/3 had triglyceride levels elevated by 11 and 14 mg/dl, respectively, compared to those with the apoE3/3 phenotype, but apoE4/4 individuals did not have elevated triglyceride levels.

The triglyceride-elevating association with the ε2 allele is consistent with the defective clearance of the apoE2-containing remnant lipoproteins that are triglyceride-rich (for review, see refs. 21 and 22). Even though these individuals lack hyperlipoproteinemia and in fact are hypocholesterolemic, they may be prone to delayed remnant lipoprotein clearance. In addition, it has been shown that apoE2 is associated with impaired lipolytic conversion of chylomicron and VLDL remnants, suggesting that apoE2 may alter lipolysis and could contribute to triglyceride accumulation (77). The effect of ε4 in elevating triglyceride levels in individuals with the apoE4/3 phenotype may also act through impaired lipolytic processing. Apolipoprotein E4 preferentially associates with VLDL (21, 22), and the quantity of this apolipoprotein on these particles may interfere with lipolysis and impair clearance of triglyceride-rich lipoproteins. On the other hand, the lack of an association of apoE4 homozygosity with elevated triglyceride levels is puzzling. The presence of two copies of apoE4 may further enrich these triglyceride-rich lipoproteins with apoE (because of the apoE4 preference for these lipoproteins) and thus enhance their catabolism.

Familial defective apoB-100, a lipid disorder causing hypercholesterolemia and increased LDL secondary to a substitution of glutamine for arginine at residue 3500 in apoB-100 (25), has been found widely distributed in several populations of the world but not in others. In two large-scale general population surveys, Bersot et al. (78),

who screened 5,160 multiracial bank employees in California, estimated the prevalence to be 1 in 1,250, and Rust, Funke, and Assmann (79), who screened 7,069 individuals in Germany, estimated the prevalence to be about 1 in 700. Innerarity et al. (25) screened 1,100 persons from the general population and from hyperlipidemia clinics and, based on adjustments for age and sex, estimated that the prevalence of FDB might be as high as 1 in 500. Other studies predict an incidence of 1 in 700 in Germany (80) and 1 in 600 in the United Kingdom (81). The highest prevalence has been reported in Switzerland. In a limited sample (three FDB cases out of 728 military recruits), the prevalence of 1 in 240 in the general population of Switzerland was estimated (82). In addition, 7 FDB cases were identified in 142 index cases from hypercholesterolemic kindreds in Switzerland.

In several studies, the defective allele for FDB generally occurs on a single apoB haplotype (83–87), suggesting that FDB in various populations resulted from the inheritance of a single mutant ancestral gene. Based on these population studies, it appears that the majority of FDB may have resulted from a founder effect arising from the Swiss-German area of present day Europe and spreading to other populations. Another apoB haplotype has been described in a German family (86) and in a Chinese man (78).

The lack of detection of the 3500 mutation in apoB-100 in 1,063 hypercholesterolemic individuals and in 1,387 individuals from the general population (Table 13) demonstrates that FDB is rare in the Turkish population and does not contribute significantly to elevated LDL-C levels in this population. In addition, the absence of FDB (3500 mutation) in the Turkish population reflects the non-European origin of this ethnic group and the lack of entry of European genes into this population. It is of interest that this mutation has not been identified in 552 hypercholesterolemic patients from Finland (88) or in 625 individuals in Israel (89). It has, however, been identified in the United States, Canada, Germany, Switzerland, the United Kingdom, the Netherlands, Italy, Austria, Denmark, and Australia (for review, see ref. 90).

In summary, the Turkish Heart Study has characterized the plasma lipids, lipoproteins, and apolipoproteins in six different regions of Turkey. The study has highlighted the groups or subpopulations at potential risk of premature coronary artery atherosclerosis. Affluence and higher education are associated with increased plasma cholesterol and LDL-C levels. In addition, the Turkish population as a whole has been found to have low HDL-C levels, which may have a genetic basis. Likewise, the increased triglyceride levels, especially in Turkish men, may further exacerbate the low HDL-C levels. It is predictable that as Turkey continues to develop, the combination of an increasing LDL-C level and a low HDL-C level represents a particularly detrimental combination that is conducive to the development of accelerated atherosclerosis. The apoE phenotype frequencies and the impact of the apoE2 and apoE4 alleles on lipid levels have been ascertained. Furthermore, a major impact of FDB on LDL-C levels has been ruled out in this population.

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