

Lecture 2

**The Turkish Heart Study:
Causes of Low HDL-C Levels**

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Publications: Turkish Heart Study: Lipids, lipoproteins, and apolipoproteins. R.W. Mahley *et al.*, *J. Lipid Res.* 36: 839–859, 1995.

New findings of the Turkish Heart Study.
R.W. Mahley *et al.*, *Türk. Kardiyol. Dern. Arş.* 30: 93–103, 2002.

Turkish Heart Study

Lipid and lipoprotein profiles of Turks

- **uniquely low levels of HDL-C**
- **possible genetic causes of low HDL-C**
- **impact of puberty on HDL-C**

Studies to determine the causes of low HDL-C in the Turkish population are ongoing.

Turkish Heart Study

Design: ~10,000 participants, volunteers
Personal questionnaires by interview
Blood sample for plasma lipids and lipoproteins

İstanbul: Large metropolitan, urban population

Adana }
Trabzon } Diets high in saturated fats (meat and dairy
Kayseri } products)

Aydın: Diets high in polyunsaturated fats (sunflower oil)

Ayvalık: Diets high in monounsaturated fats (olive oil)

Six Regions Surveyed for Turkish Heart Study



Age-adjusted Plasma Cholesterol Levels Mean (mg/dl)

	Turkish Men	Turkish Women
İstanbul	202	181
Adana	184	190
Trabzon	174	175
Kayseri	171	179
Aydın	173	166
Ayvalık	160	162

Ideal cholesterol level: less than 200 mg/dl.

The plasma cholesterol levels were different in the various regions.

- Highest: Istanbul men.
- Also high in areas where saturated fats are consumed.

The lowest levels were seen in Ayvalık, where consumption of mono-unsaturated fats is common.

Turks Tend To Have Low Total Cholesterol Levels

Total cholesterol < 200 mg/dl

- **66% of men**
- **78% of women**
- **95% of Turks in Ayvalık**

Total cholesterol > 200 mg/dl

- **50% of affluent men and women**

Age-adjusted Plasma HDL-C Levels Mean (mg/dl)

	Turkish Men	Turkish Women
İstanbul	38	45
Adana	34	39
Trabzon	34	42
Kayseri	34	37
Aydın	37	43
Ayvalık	38	42
U.S. Mean	~47	~56

Ideal HDL-C: greater than 35 mg/dl.

The most important observation: Turks have very low HDL-C levels.

Plasma Levels of HDL Cholesterol (mg/dl)

	Men	Women
Belgium	~45	~60
Germany	47	60
U.S.A.	46	55
Tibet	~55	~55
Japan	55	55
China	~45	~45
Korea	45	45
Turkey	34–37	37–43

Turks tend to have the lowest HDL-C levels seen in any population in the world.

- 10–15 mg/dl lower than those seen in Europe or the United States.

Mean HDL-C Levels (mg/dl)

	Americans in U.S.A.	Germans in Germany	Turks in Turkey	Turks in Germany	Turks in S.F.
Men	47	47	37	38	37
Women	56	60	43	45	46

Turks living outside of Turkey also have low HDL-C levels. This strongly suggests that genetics is playing a major role in determining the HDL-C levels.

Total Cholesterol/HDL Cholesterol Ratio

Ideal: ≤ 3.5

High Risk: > 4.5

	Americans in U.S.A.	Germans in Germany	Turks in Germany	Turks in İstanbul	Turks in Ayvalık
Men	4.5	4.6	5.1	5.5	4.3
Women	3.8	3.5	3.8	4.2	3.9

Conclusion: Highest ratios seen in Turkish men and women in İstanbul.

HDL-C Is Abnormally Low in the Turkish Population

	HDL-C < 35 mg/dl	HDL-C < 40 mg/dl
Turkish Men	53% (15% of U.S. men)	74%
Turkish Women	26% (5% of U.S. women)	53%

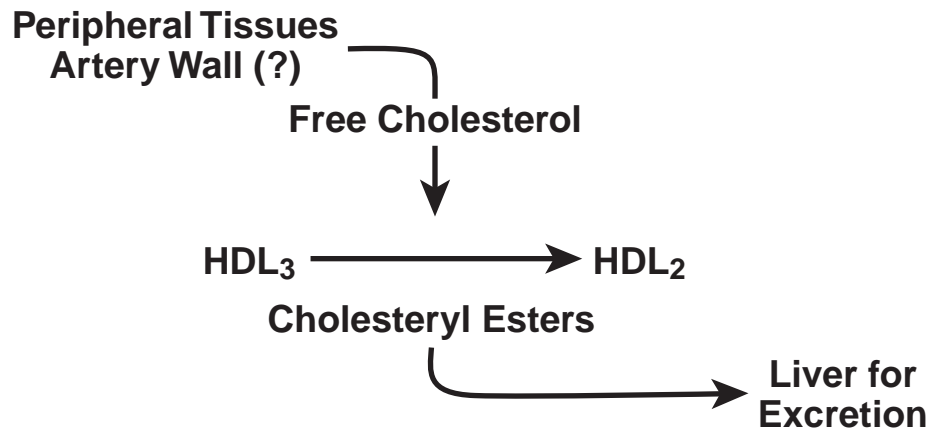
New NCEP guidelines indicate that HDL-C levels <40 mg/dl are a CHD risk factor.

Low HDL-C in Turks

- appears to be largely genetic
- not accounted for by common environmental factors
 - smoking
 - lack of physical activity
 - obesity
 - diabetes
 - diets that raise triglycerides
- genetics + environment → ↓↓HDL-C (worse)

Genetics and environment are undoubtedly working in concert to make the low HDL-C an even worse problem in Turks.

Role of HDL in Cholesterol Metabolism



HDL transports cholesterol from cells with excess cholesterol, including cholesterol-loaded cells of the artery wall, to the liver where the cholesterol is excreted from the body.

Causes of Low HDL-C Levels

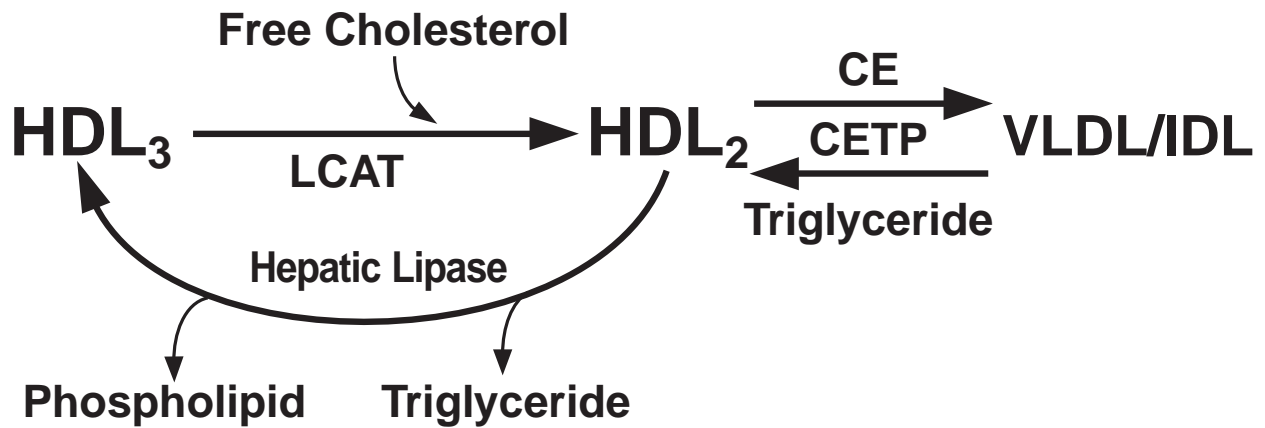
↑ hepatic lipase

↓ lecithin:cholesterol
acyltransferase

↑ cholesteryl ester
transfer protein

↓ apoA1 production

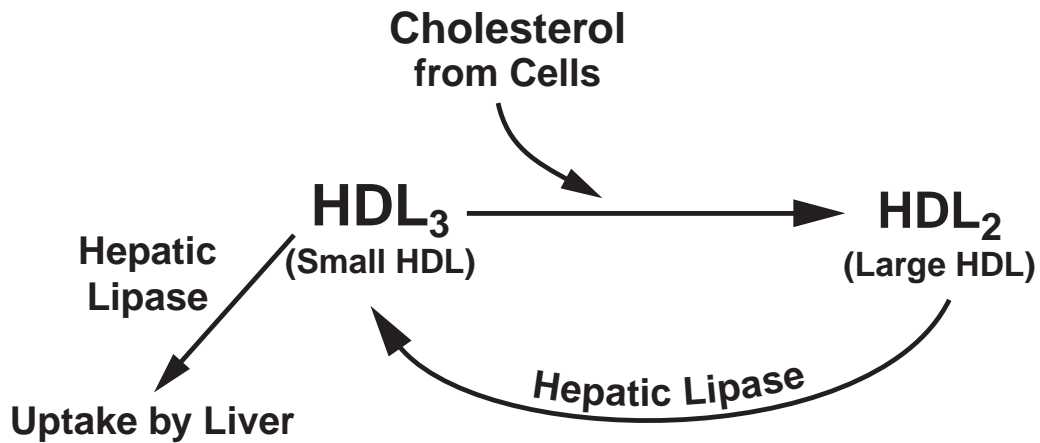
↓ lipoprotein lipase



High levels of some factors cause low HDL-C levels whereas high levels of others cause low HDL-C.

Hepatic Lipase (HL)

Enzyme on the surface of liver cells that hydrolyzes lipids on the lipoproteins and facilitates the uptake of the lipoprotein particles.



Net Effect of Increased HL: • Decreased HDL Levels

Elevated levels of hepatic lipase have been shown to occur commonly in the Turkish population.

Hepatic Lipase Activity Is Increased in Turks with Low HDL Levels

↑ Hepatic lipase (25–30%):

• ↓ HDL-C	↓ HDL ₂		↓ LpAI	
	Non-Turkish (White Americans)		Turkish (Istanbul)	
	Men	Women	Men	Women
HDL-C (mg/dl)	45	58	37	43
Hepatic Lipase (mmol/h/l)	38	26	48	35

Bersot et al., J. Lipid Res. 40:432, 1999;
Mahley et al., J. Lipid Res. 41:1290, 2000

Elevated levels of hepatic lipase are associated with low levels of HDL-C in Turks.

HDL Subfraction Quantitation Study in Turks

- Ultracentrifugation: HDL₂, HDL₃
- Differential precipitation: HDL₂, HDL₃
- Electroimmunoassay: LpAI, LpAI/AII
 - 196 men HDL-C = 36 ± 8 mg/dL
 - 210 women HDL-C = 42 ± 9 mg/dL

Several methods have been used to isolate and characterize the HDL subfractions.

HDL₂ \approx LpAI

HDL₃ \approx LpAI/AII

Comparison of HDL Subclasses in the Turks and French

	<i>n</i>	HDL-C	Total ApoAI	LpAI mg/dl	LpAI/All
Turkish					
Men	196	36 ± 8	122 ± 19	39 ± 9	84
Women	210	42 ± 9	123 ± 19	48 ± 13	76
French^a					
Men	40	59 ± 15	120 ± 20	49 ± 9	71
Women	45	65 ± 14	133 ± 17	60 ± 13	73

^aParra *et al. Clin. Chem.* 36: 1431, 1990.

Electroimmunoassay demonstrates that Turks have significantly lower levels of LpAI and elevated levels of LpAI/All compared with French men and women.

Characterization of HDL in Turks

Low HDL-C levels

- HDL₂ decreased
 - LpAI decreased
 - LpAI/AII increased
- } Proatherogenic changes in HDL

Plasma Triglyceride Levels Mean (mg/dl)

	Turkish Men	Turkish Women
İstanbul	142	90
Adana	145	109
Trabzon	129	95
Kayseri	128	121
Aydın	143	95
Ayvalık	124	112

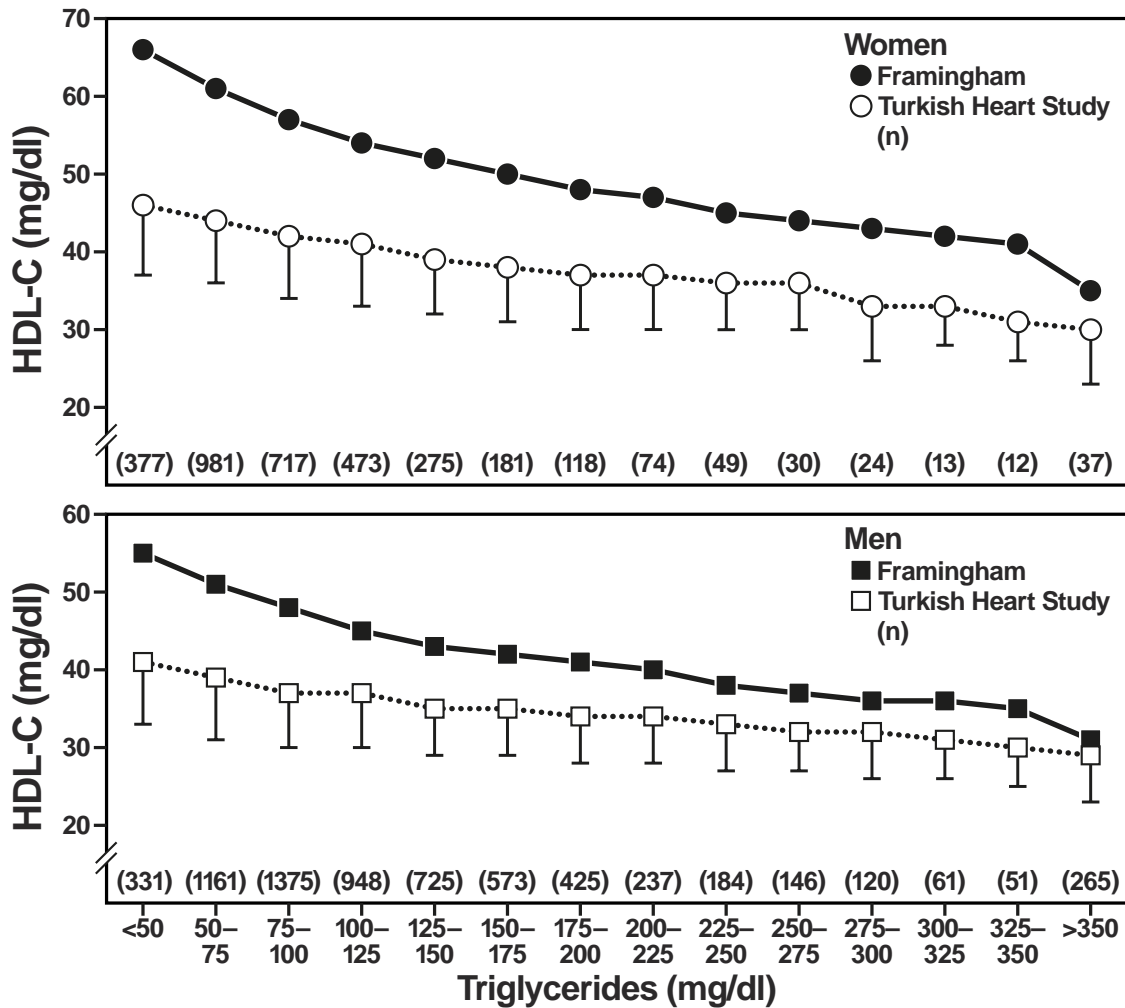
Ideal triglyceride level: less than 200 mg/dl.

Conclusion: Plasma triglyceride levels tend to be higher than seen in many other populations.

It is well known that there is an inverse relationship between triglycerides and HDL-C. However, elevated triglycerides do not explain the low HDL-C levels seen in Turks.

HDL-C versus Triglycerides

Comparison of Framingham and Turkish Heart Study Data



1. HDL-C levels are lower in Turks at all triglyceride levels. In Turkish women, the HDL-C difference is ~20 mg/dl at lower triglyceride levels and ~10 mg/dl at higher levels. In Turkish men, the differences in HDL-C range from 15 to 15 mg/dl.
2. The magnitude of the impact of triglycerides on HDL-C is significantly greater in U.S. men and women—the inverse relationship is steeper.

Differences Between Mean HDL-C (mg/dl) at Low versus Higher Triglyceride Levels

Triglycerides (mg/dl)	Framingham Study		Turkish Heart Study	
	Males	Females	Males	Females
<50	55	66	42	46
200–225	39	46	34	34
300–325	36	42	32	32

	}	16	}	20	}	8	}	9	}	12
	}	19	}	24	}	10	}	34	}	32

Looking at U.S. men and women, we can see that HDL-C levels decrease by 19–24 mg/dl comparing triglyceride levels of <50 versus 300–325 mg/dl (men, 55→36 mg/dl; women, 66→42 mg/dl).

In Turks, on the other hand, HDL-C levels drop 10–12 mg/dl (men, 42→32 mg/dl; women, 46→32 mg/dl). In fact, HDL-C levels in Turkish men and women are virtually identical (32 mg/dl) at triglyceride levels of 300–325 mg/dl. This loss of gender differences is unique to the Turks.

Gender-specific Differences in Mean HDL-C Levels at Increasing Triglyceride Concentrations in U.S. versus Turkish Men and Women

Triglycerides (mg/dl)	Framingham Study	Turkish Heart Study	
	HDL-C Differences M vs. W (mg/dl)	HDL-C Differences M vs. W (mg/dl)	<i>p</i> M vs. W
<50	11	4	<0.0001
100–125	9	4	<0.0001
225–250	7	3	0.009
250–275	7	4	0.0003
275–300	7	1	NS
300–325	6	2	NS
325–350	6	1	NS

In Turks, the gap in HDL-C levels between men and women is narrow even at low triglyceride levels (4 mg/dl). At higher triglyceride levels (>275 mg/dl) the difference in HDL-C level between Turkish men and women is lost. These gender differences are very interesting. High triglycerides negate the gender differences in HDL-C in Turks. There may be a basal hormonal difference in Turks that contributes to some of the unique lipid and lipoprotein characteristics.

Elevated Hepatic Lipase Associated with Low HDL-C in the Turkish Population —Postulates—

Primary defect

- promoter polymorphism causing increased hepatic lipase production

Secondary to hormonal modulation

- androgen/estrogen balance altering hepatic lipase synthesis/secretion/activation
- testosterone known to regulate hepatic lipase gene expression

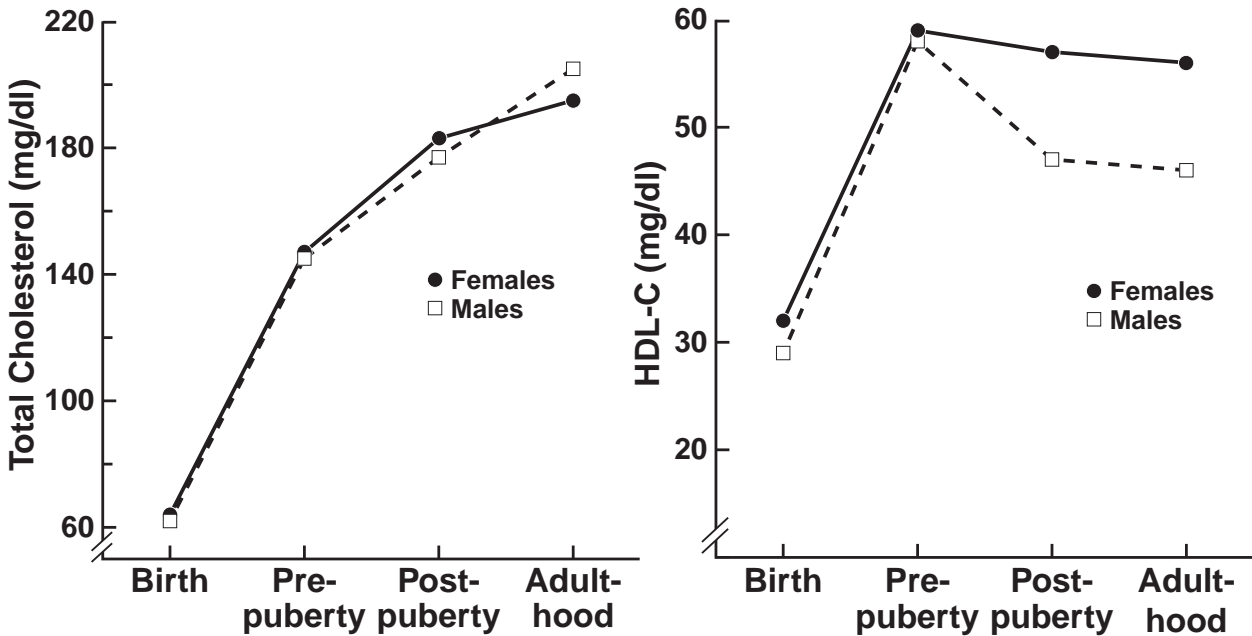
The possible role of hepatic lipase associated with low HDL-C in Turks may be related to hormonal differences.

Do Turks Have Low HDL-C Levels Throughout Life—Both Pre- and Postpuberty?

- **profound hormonal changes at puberty**
- **significant changes in plasma lipids, including HDL-C, during childhood and postpuberty**
- **comparison of changes in HDL-C at birth, prepuberty, and postpuberty in western Europeans/Americans versus Turks**

The following studies are designed to determine if Turks have a constant state of low HDL-C from birth throughout adulthood.

Typical Changes in Total Cholesterol and HDL-C with Age in Western European/U.S. Populations



Birth: plasma total cholesterol and HDL-C levels are low; no gender differences.

Prepuberty: total cholesterol and HDL-C levels increase; HDL-C levels are typically 55–60 mg/dl; no gender differences.

Post-puberty: total cholesterol levels continue to increase; HDL-C levels decrease ~10 mg/dl in males and 2–3 mg/dl in females.

What happens in Turks?

Turkish Heart Study: Cord Blood Study in Turkish Newborns

**105 male and female newborns delivered by
cesarean section at the American Hospital
(Istanbul)**

All normal deliveries and healthy newborns

Cord blood samples analyzed for plasma lipids

Cord Blood Study in Turkish Newborns

	Number (<i>n</i>)	Gestation (weeks)	Weight (kg)	Length (cm)	Body Mass Index (kg/m ²)
Males	68	38 ± 2	3.27 ± 0.52	50 ± 2	14 ± 1
Females	37	38 ± 2	3.24 ± 0.44	49 ± 2	14 ± 1
	Total Cholesterol (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)		Triglycerides (mg/dl)
Males	56 ± 15 (34–98)	23 ± 9	29 ± 9 (11–52)		24 ± 9 (10–46)
Females	61 ± 18 (37–124)	24 ± 10	32 ± 12 (15–68)		23 ± 9 (11–42)

Total cholesterol and HDL-C levels are low at birth and similar to what is seen in European/U.S. populations.

Turkish Heart Study: School Children's Study (8- to 10-year-old boys and girls)

Children:

- 225 children (103 boys; 122 girls)
- two schools in Ankara
 - upper socioeconomic community (Çankaya İlçesindeki; $n = 82$)
 - lower socioeconomic community (Keciören İlçesindeki; $n = 143$)

Design:

- biodata forms filled out at home
- diet history and recall recorded
- fasting blood sample obtained

Joint Project:

- Gladstone Institute (American Hospital, İstanbul) and Hacettepe University (Dietetics School)

Children's Study Participants

Gladstone Institute

R. W. Mahley, M.D., Ph.D.

T. P. Bersot, M.D., Ph.D.

Guy M. Pépin

Ayşen Ağaçdiken, M.D.

Zümrüt Algan, M.D.

Erhan Palaoglu, Ph.D.

Hacettepe University

Prof. Dr. Perihan Arslan

Prof. Dr. Gülden Pekcan

Prof. Dr. Nilgün Kavaağoglu

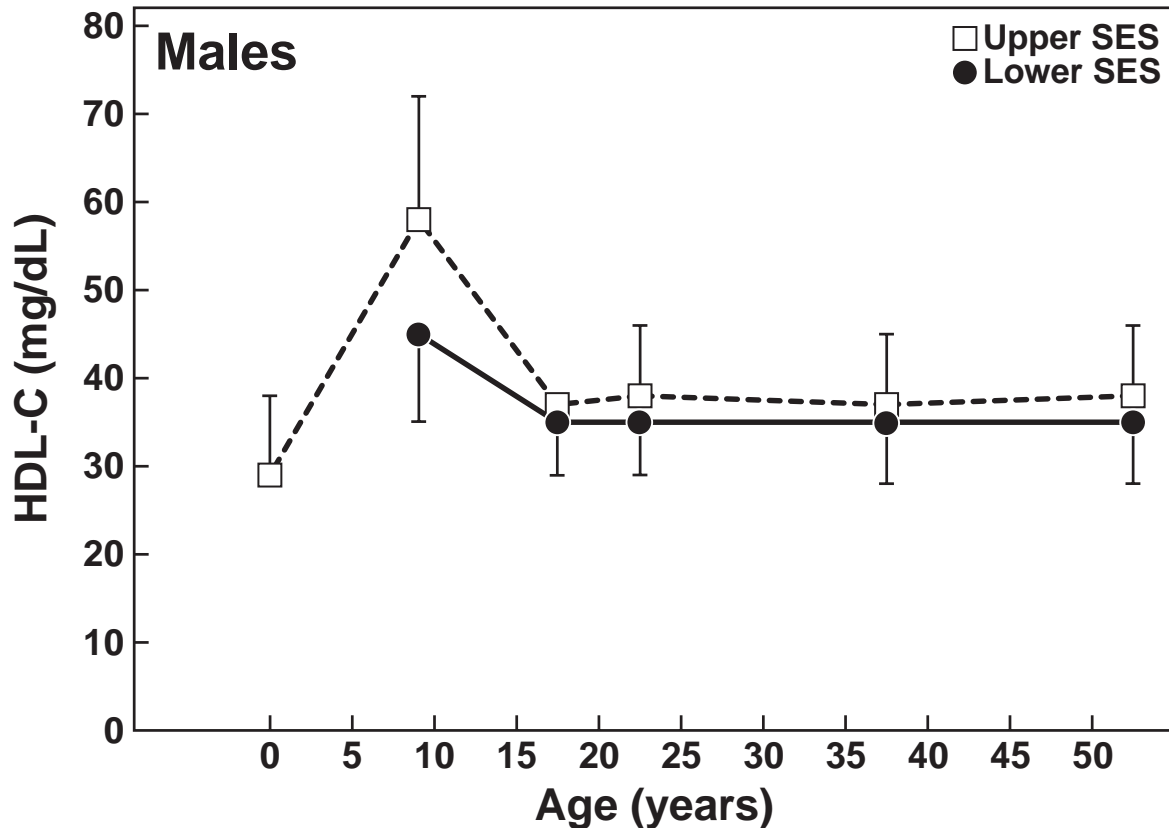
Asst. Prof. Neşlişah Rakicioğlu

Berat Nursal

**Plasma Lipid Levels (mean mg/dl \pm S.D.):
Upper and Lower Socioeconomics
(8–10 Years of Age)**

	Total Cholesterol		HDL-C	
	Boys	Girls	Boys	Girls
Total	138 \pm 26	143 \pm 26	50 \pm 13	49 \pm 12
Upper Socio- economics	154 \pm 20	158 \pm 25	58 \pm 14	55 \pm 12
Lower Socio- economics	129 \pm 25	134 \pm 22	45 \pm 10	45 \pm 10

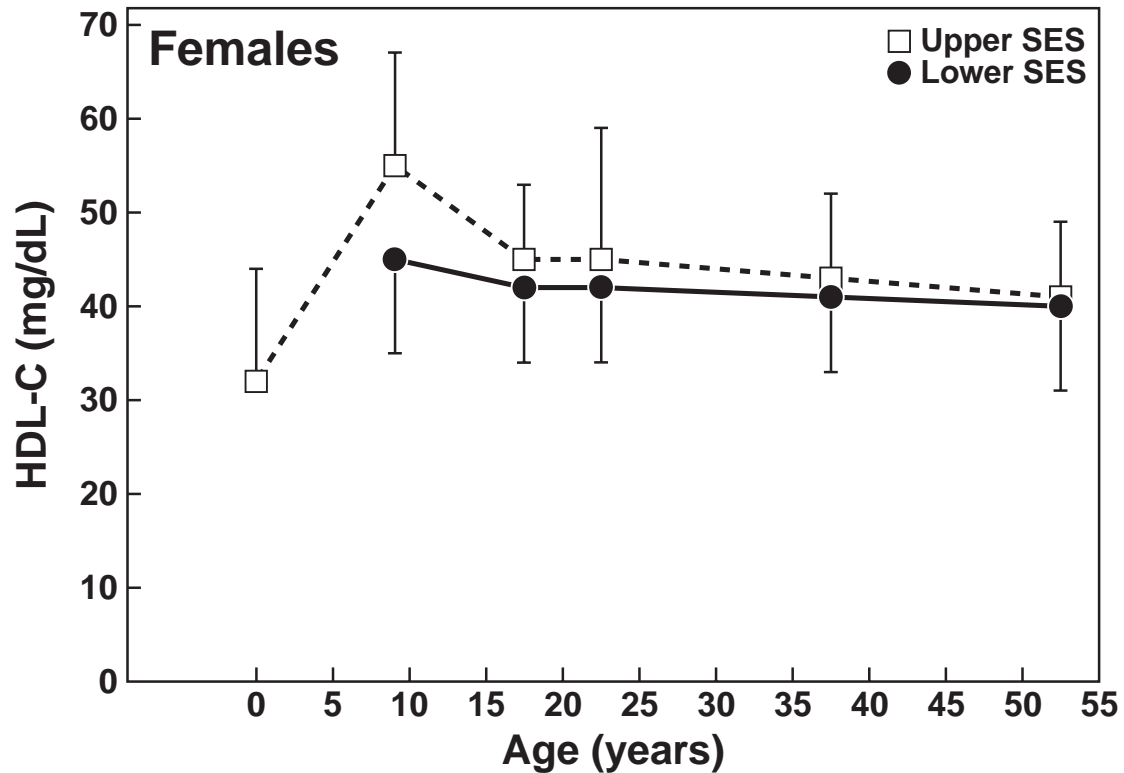
Socioeconomic Status (SES) in Turkey Affects HDL-C Levels Only Before Puberty



Shown here are the HDL-C levels in Turkish males (upper and lower SES) from birth to 55 years of age. Post-puberty the levels drop dramatically: ~20 mg/dl (upper SES) or ~10 mg/dl (lower SES).

HDL-C levels remain constant throughout adult life and SES plays no role after puberty.

Socioeconomic Status (SES) in Turkey Affects HDL-C Levels Only Before Puberty



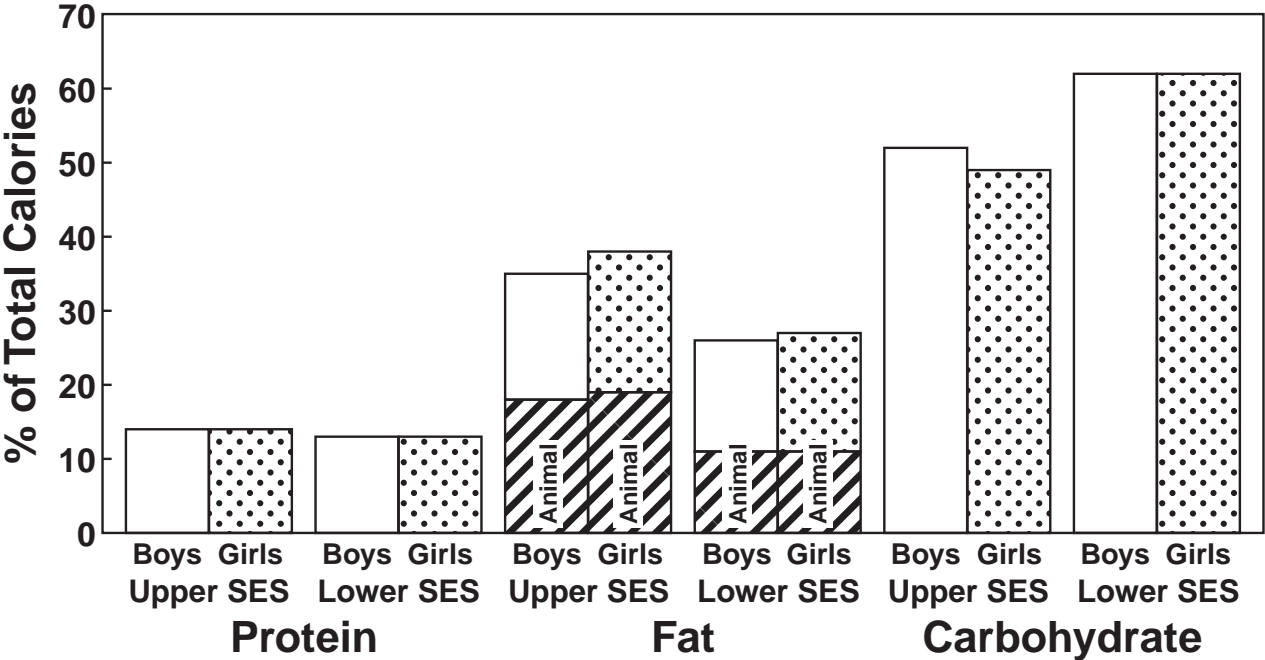
The upper SES girls have higher HDL-C levels, and they drop post-puberty to adult levels: ~13 mg/dl decrease.

The adult HDL-C levels in women are constant and unaffected by SES.

Why Was There Such a Difference Between the Upper and Lower Socioeconomic Status Children?

- **effect of dietary differences on HDL-C levels in the 8–10-year-old prepubescent Turkish boys and girls**

Dietary Intake for 8–10-year-old Turkish Children

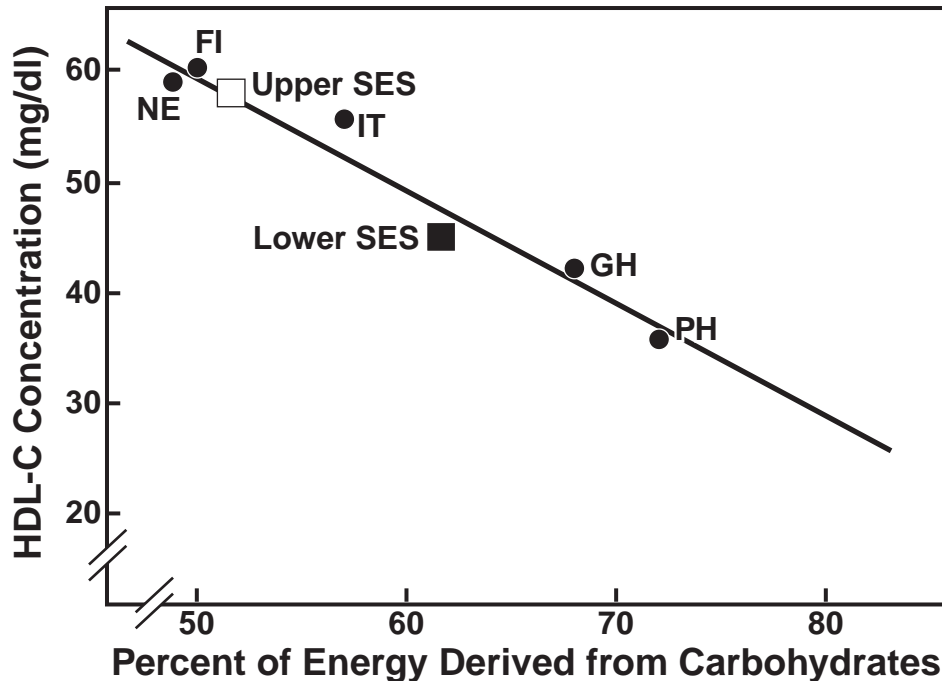


Protein calories: ~13% of calories in all children.

Fat calories: ~36% in the upper SES children (increased animal saturated fat especially) versus 26% in the lower SES children; increased fat correlates with increased plasma cholesterol levels in the upper SES children.

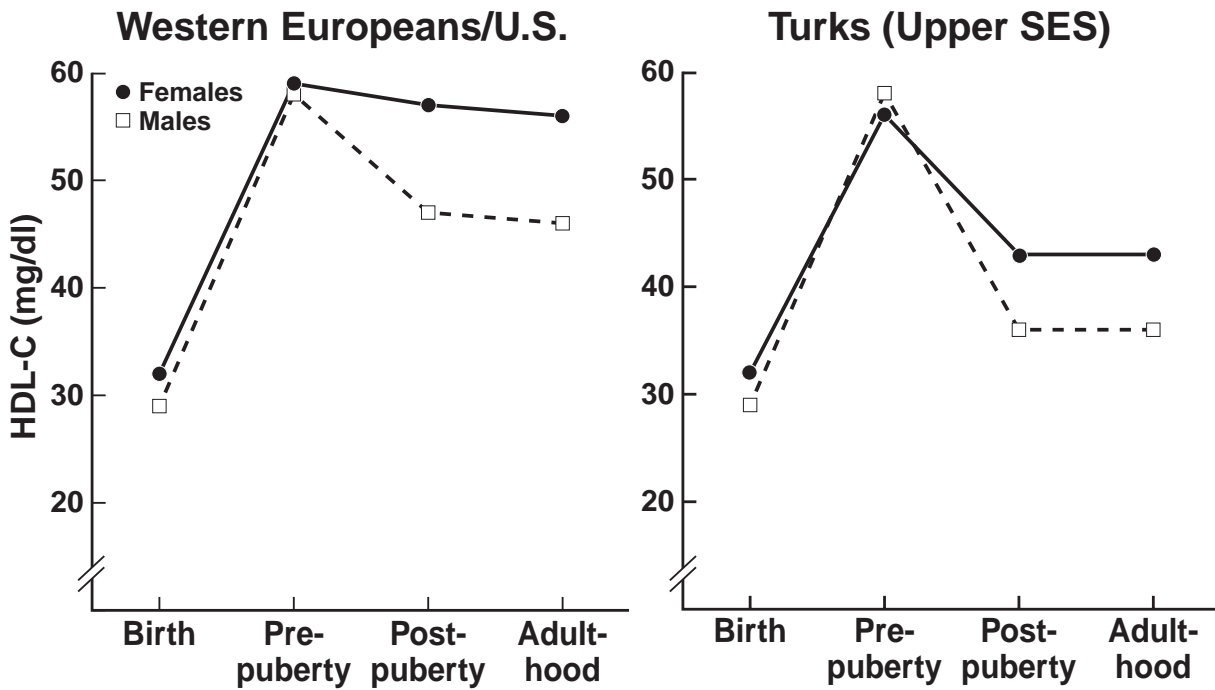
Carbohydrate calories: ~62% in the lower SES children versus ~50% in the upper SES group. Higher carbohydrate consumption associated with lower HDL-C levels.

Dietary Carbohydrate Intake Correlates Inversely with HDL-C Levels (8–10-year-old Boys)



Dietary carbohydrates correlate inversely with HDL-C levels in 8–10-year-old boys around the world. Diets in prepubescent boys in Finland (FI) and the Netherlands (NE) contain about 50% of calories from carbohydrates. This is similar to the results obtained for the Turkish boys in the upper SES group, and the HDL-C levels in the European and Turkish boys are virtually identical (55–60 mg/dl). However, the lower SES boys consuming higher quantities of carbohydrates had lower HDL-C levels (~45 mg/dl). This value is predicted by the regression line as shown (Knuiman *et al.*, *Arteriosclerosis* 7:612, 1987).

Changes in HDL-C Levels with Age



There is a dramatic difference in the magnitude of the decrease in HDL-C levels post-puberty in the western European versus Turkish males and females. Values for the Turkish children are those from the upper SES groups since the diet of this group closely resembles the European diets with respect to carbohydrate and fat.

Possible Factors Modulating HDL-C Levels at Puberty

Hormonal differences occurring at puberty

- different ethnic and genetic backgrounds
- involve androgens, estrogens, leptin, etc.

Ethnic differences in plasma levels

- Turks may have low levels of sex hormone binding globulin (\downarrow SHBG = \uparrow free, bioactive testosterone)
- \uparrow testosterone = \uparrow hepatic lipase = \downarrow HDL-C

Turkish Heart Study

- I. Unique lipid and lipoprotein profile**
 - very low levels of HDL-C
- II. Possible genetic cause of low HDL-C**
 - elevated levels of hepatic lipase
- III. Characterization of HDL fraction**
 - low levels of HDL₂ and LpAI
- IV. Dramatic decrease in HDL-C post-puberty in Turks**
 - hormonal changes at puberty involving androgen, estrogen, leptin, etc.

Low HDL-C: A Powerful CHD Risk Factor

HDL-C (mg/dl)	Total Cholesterol (mg/dl)	% Incidence Rates for CHD
<40	<200	11.2
>60	<200	3.8
<40	>260	12.5
50–59	>260	9.0
>60	>260	3.8

Framingham Study

There are two factors that must be taken into account: total plasma cholesterol and HDL-C. A powerful predictor of CHD risk is the total cholesterol/HDL-C ratio.

Total Cholesterol/HDL Cholesterol Ratio

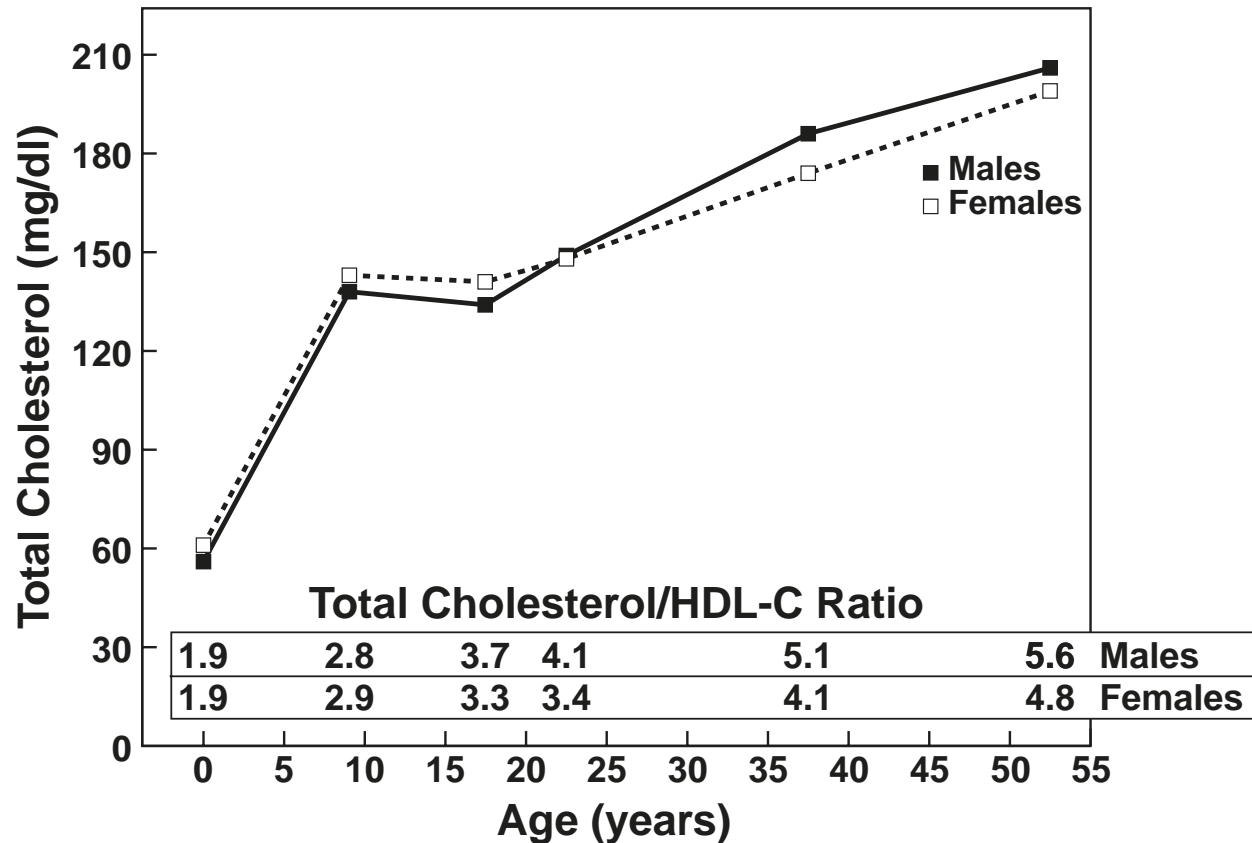
Ideal: ≤ 3.5

High Risk: > 4.5

	Americans in USA	Germans in Germany	Turks in Germany	Turks in İstanbul	Turks in Ayvalık
Men	4.5	4.6	5.1	5.5	4.3
Women	3.8	3.5	3.8	4.2	3.9

Conclusion: Highest ratios seen in Turkish men and women in İstanbul.

Plasma Total Cholesterol: Birth to 54 Years (Total Cholesterol/HDL-C Ratio; Males versus Females)



The total cholesterol/HDL-C ratio is low at birth and pre-puberty but begins to rise after the age of 20.

Question

Is a low HDL-C a risk factor in the presence of a low or normal cholesterol level (ideal < 200 mg/dl)?

A 10–15 mg/dl lower HDL-C in the Turkish population may suggest that an ideal cholesterol level is lower than 200 mg/dl.

Total Cholesterol/HDL-C Ratios:

Ideal, ≤ 3.5 ; High Risk, > 4.5

$$\frac{180}{30} = 6.0 \quad \frac{180}{40} = 4.5 \quad \frac{180}{50} = 3.6$$

$$\frac{200}{30} = 6.6 \quad \frac{200}{40} = 5.0 \quad \frac{200}{50} = 4.0$$

In the context of low HDL-C, the total cholesterol level must be significantly lower than 200 mg/dl.

HDL-C Is Abnormally Low in the Turkish Population

	HDL-C < 35 mg/dl	HDL-C < 40 mg/dl
Turkish Men	53% (15% of U.S. men)	74%
Turkish Women	26% (5% of U.S. women)	53%

New NCEP guidelines indicate that HDL-C levels <40 mg/dl are a CHD risk factor.