M E D I C A L G R A N D S R O U N D S

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High Density Lipoprotein (HDL): Physiology, Pathophysiology, and Relationship to Atherosclerosis

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I. Introduction

In 1951, Barr and associates reported a relative and absolute reduction in alpha lipoprotein levels in patients with atherosclerosis (1). This observation was confirmed repeatedly (2-9) but investigative attention was focussed primarily on the high levels of beta lipoprotein rather than the low alpha lipoprotein levels found in patients with atherosclerosis.

In 1975, alpha lipoprotein or high density lipoprotein (HDL) as it by then was known, was "rediscovered" by Miller and Miller (10). These authors suggested that low Plasma HDL levels might be associated with atherosclerosis and cited several pieces of circumstantial evidence to support their suggestion:

1) Tissue cholesterol pools, measured by isotope dilution in 8 patients, were negatively correlated with plasma HDL cholesterol concentrations (Figure 1) (11).

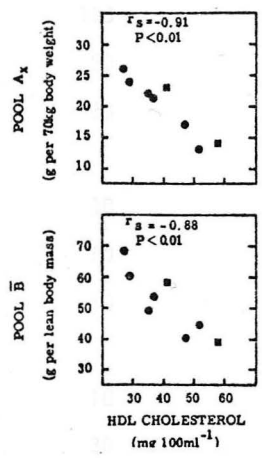

Figure 1. Relationship between HDL-cholesterol concentration and body cholesterol pool size in 8 subjects. Pool A_x is a rapidly exchanging tissue pool while pool $\bar{B}$ is a slowly exchanging tissue pool. ● = males, ■ = females (11).

2) Hypercholesterolemia is inversely correlated with plasma HDL-cholesterol levels.

3) Hypertriglyceridemia is inversely correlated with plasma HDL-cholesterol levels.

4) Other risk factors for premature heart disease such as obesity, diabetes mellitus, physical inactivity, and male sex are commonly associated with reduced HDL cholesterol concentrations.

5) Patients with clinical ischemic heart disease have significantly lower HDL-cholesterol concentrations than healthy subjects within the same community.

Additional evidence supporting the notion that elevated HDL levels were not atherogenic and might actually protect against atherosclerosis came from comparative biochemical studies in other mammalian species.

In most species other than man, most of the plasma cholesterol is in the form of HDL (Table 1) (12,13). In humans, on the other hand, up to 70% of the plasma cholesterol is carried in LDL while HDL contributes only a minor fraction (17-20%). This human lipoprotein profile, with LDL predominating at the expense of HDL, is thought to play a major role in our susceptibility to atherosclerosis.

Table 1. Concentration of Plasma Lipoproteins in Mammals+

	VLDL	LDL	HDL
		mg/dl*	
Primates			
Man	100	200-400	300
Baboon	20	120	350
Rhesus Monkey	30	200	400
Carnivores			
Dog	30	70	750
Harbor Seal	50	150	1,200
Dolphin	50	80	350
Ruminants			
Bovine	10	20	800
Bison	30	30	200
Herbivores			
Rabbit	100	60	350
Guinea Pig	50	180	10
Hamster	100	100	400
Omnivores			
Rat	80	30	400
Pig	100	180	300

* Total mass of lipoprotein derived from analytical ultracentrifugation. The values would differ somewhat if expressed in terms of lipoprotein-cholesterol.

\+ From Ref. 12.

Since 1975, HDL has been the focus of enormous experimental attention. The inverse correlation of HDL-cholesterol levels with premature heart disease risk has been reaffirmed and the experimental evidence has been embellished and widely publicized. HDL levels are being measured in all sorts of groups from long-distance runners to renal dialysis patients. The practices of groups found to have high HDL levels are sometimes embraced as measures which promote health and prolong life. An example of such thinking can be found in the June, 1979 issue of *Runners World*. On the front cover, an article is featured with the headline "Heart Disease Can be Prevented by Running". The article inside is entitled, "Running Away from Heart Disease: Becoming Familiar With Your Lipoproteins Could Save Your Life". The genesis of this claim relates to the finding that people who run more than 15 miles per week have higher HDL- and lower LDL-cholesterol levels than do more sedentary individuals (14). Unfortunately, the implication that running will save or prolong life by raising HDL-cholesterol levels or by any other means is not supported by fact. (Incidently, the article in *Runners World* is reasonably factual - only the title is misleading.)

As we shall see, the current HDL story is neither simple nor complete. The purpose of the following discussion is to review the evidence that propelled HDL into the cardiac risk factor limelight and to judge if the measurement of the cholesterol in this lipoprotein has significant clinical usefulness at the present time.

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6) Dodds, C. and G.L. Mills. Influence of Myocardial Infarction on Plasma-Lipoprotein Concentration. *Lancet* 1:1160-1963, 1959.

7) Carlson, L.A. Serum Lipids in Men with Myocardial Infarction. *Acta Med. Scand.* 167:399-413, 1960.

8) Keys, A. and F. Fedanza. Serum Cholesterol and Relative Body Weight of Coronary Patients in Different Populations. *Circulation* 22:1091-1106, 1960.

9) Medalie, J.H. et al. Five-Year Myocardial Infarction Incidence: II. Association of Single Variables to Age and Birth Place. *J. Chron. Dis.* 26:329-349, 1973.

10) Miller, G.J. and N.E. Miller. Plasma-High-Density-Lipoprotein Concentration and Development of Schaemic Heart-Disease. *Lancet* 1:16-19, 1975.

11) Miller, N.E. et al. Relationships between plasma lipoprotein cholesterol concentrations and the pool size and metabolism of cholesterol in man. *Atheros.* 23:535-547, 1976.

12) Havel, R.J. Lipids and Atherosclerosis. *Baylor University Cardiovascular Research Bulletin* 15:93-97, 1977.

13) Zilversmit, D.B. et al High Plasma Cholesterol in Mink (Mustela vison) Without Atherosclerosis. *Atheros.* 26:97-102, 1977.

14) Wood, P.D. et al. The Distribution of Plasma Lipoproteins in Middle-aged Male Runners. *Metab.* 25:1249-1257, 1976.

II. HDL as a Risk Factor for Cardiovascular Disease: Epidemiologic and Clinical Data.

It was noted in the introduction that low HDL levels were observed in patients with atherosclerosis in 1951. Within the last 4 years, however, a series of studies employing modern lipoprotein techniques have been published and all seem to underscore the importance of HDL-cholesterol as an indicator or risk for cardiovascular disease. These studies are both prospective (incidence studies) and retrospective (prevalence studies) and encompass relatively large groups from divergent backgrounds. A summary of each of the more important studies is given below:

1. Honolulu Heart Study (15). In this prevalence study, a cohort of men (n=8006) of Japanese ancestry born in the years 1900-1919 and living on the island of Oahu in 1965 were evaluated. Serum lipoproteins were measured in 1970-1972 on a large sub-sample of the original group. Later, a number of smaller groups were called in for repeat evaluation, including a 30% probability sample and a group who had developed coronary heart disease (myocardial infarction, acute coronary insufficiency or angina pectoris) since the start of the study. Lipoprotein determinations are performed on fasting plasma specimens using standard techniques. Lipid values for the men with and without coronary heart disease (CHD) (1970-1972) are listed in Table 2.

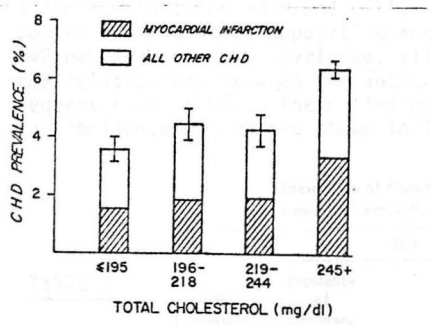
Table 2. Lipid Values for Fasting Men with and without Coronary Heart Disease (CHD) (1970-1972).

Group	Cholesterol Fraction (Mg/Dl)				Triglyceride (Mg/Dl)
	Total	Alpha	Beta	Pre-Beta	
50-59 yr of age:					
All CHD (141)*	228.1†	41.8‡	151.4‡	34.9	187.3
Myocardial infarction only (70)	228.2	40.5‡	151.2†	36.5	200.7
Control (1137)	220.7	44.6	142.1	34.1	180.3
≥60 yr of age:					
All CHD (123)	231.1‡	42.0‡	155.9‡	33.2	165.9
Myocardial infarction only (58)	233.5†	43.1	157.6†	32.8	155.4
Control (618)	220.9	46.2	144.4	30.3	158.7

*No. of cases.
†Significantly different from control group ($P < 0.05$) by 2-tailed t-test.
‡Significantly different from control group ($P < 0.01$) by 2-tailed t-test.

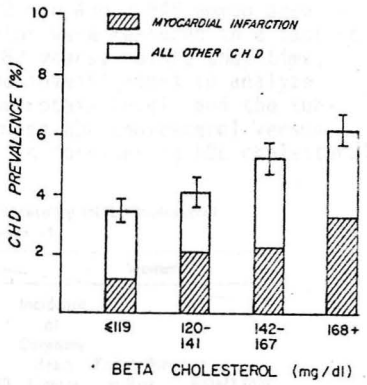
Table 2

As in previous studies, these investigators found that the prevalence of CHD increased with either the total plasma cholesterol (Fig. 2) or the plasma beta cholesterol (LDL) level (Fig. 3). When the prevalence of CHD was compared to the plasma HDL cholesterol (alpha cholesterol) levels, those groups of men with



Estimated Prevalence of Coronary Heart Disease (CHD) According to Total-Serum-Cholesterol Quartiles.
Bars indicate the standard error.

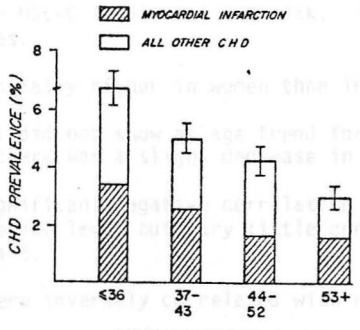
Figure 2.



Estimated Prevalence of Coronary Heart Disease (CHD) According to Beta-Cholesterol Quartiles.
Bars indicate the standard error.

Figure 3.

the lowest HDL-cholesterol levels had the highest prevalence of CHD while the lowest prevalence of CHD was found in the groups with the highest HDL levels (Fig. 4).



Estimated Prevalence of Coronary Heart Disease (CHD) According to Alpha-Cholesterol Quartiles.
Bars indicate the standard error.

Figure 4.

Thus while the average HDL cholesterol level did not differ greatly in magnitude between those groups with and without CHD (Table 2), the prevalence of CHD did vary considerably when examined over the entire range of HDL levels measured (Fig. 4).

2. Framingham Study (16). This study has been in continuous operation since 1949. The initial enrollment included 2,282 men and 2,845 women aged 30 to 62. Between 1969 and 1971, lipoproteins were measured in a fasting cohort of this study population aged 49 to 82 years. Since that time, 142 new cases of CHD developed, allowing the investigators to analyze the relationship between the antecedent lipoprotein levels and the subsequent risk of CHD. Their findings concerning HDL cholesterol versus the incidence of CHD are shown in Table 3. In considering HDL cholesterol

Table 3. Incidence of Coronary Heart Disease by HDL Cholesterol Level—Framingham Study, Exam 11

HDL Cholesterol Level (mg/dl)	Men			Women		
	Incidence of Coronary Heart Disease	Population at Risk	Rate/1,000	Incidence of Coronary Heart Disease	Population at Risk	Rate/1,000
All levels	79	1,025	77.1	63	1,445	43.6
<25	3	17	176.5	0	4	0.0
25–34	17	170	100.0	11	67	164.2
35–44	35	335	104.5	12	220	54.5
45–54	15	294	51.0	19	386	49.2
55–64	8	134	59.7	14	353	39.7
65–74	1	40	25.0	3	216	13.9
75+	0	35	0	4	199	20.1

NOTE: The majority of persons were followed for four years. However, a small number may have been followed for as few as two years or as many as eight years.

values between 25 and 74 mg/dl. These investigators observed an inverse correlation between HDL-C levels and CHD risk. They also noted the following correlated variables.

i. HDL was substantially higher in women than in men.

ii. The HDL-C level did not show an age trend for men (between 49 and 82 years) but in women, there was a slight decrease in the level from ages 50 to 80.

iii. There was a significant negative correlation of the HDL-C level with the plasma triglyceride level but vary little correlation with either LDL-C or total plasma C.

iv. HDL-C levels were inversely correlated with relative body weight.

v. Weak negative correlations were found with glucose tolerance and systolic blood pressure but none with cigarette smoking.

On the basis of this analysis, the authors concluded that an obese person with glucose intolerance or a high triglyceride level is more likely to have a low HDL-C level than a high one. As we shall see later, their conclusions regarding cigarette smoking may not apply to younger age groups.

In attempts to determine which data on plasma lipids and lipoproteins would be most predictive of CHD risk, the authors calculated likelihood ratios for various lipid profiles (Table 4). The higher the value for the ratio,

Table 4. Likelihood Ratios for Various Lipid Profiles of Coronary Heart Disease—Framingham Study, Exam 11

Lipid	Men	Women
HDL cholesterol	14.03*	21.21*
LDL cholesterol	4.39†	4.53†
Triglyceride	0.51	9.52‡
Total cholesterol	1.98	2.26
HDL cholesterol:total cholesterol	17.11*	20.41*
LDL and total cholesterol, triglyceride	8.26†	19.69*
HDL and total cholesterol, triglyceride	19.19*	24.21*
HDL and LDL cholesterol, triglyceride	18.90*	24.73*
HDL and LDL cholesterol	18.66*	23.70*
HDL cholesterol:total cholesterol, LDL cholesterol	17.16*	20.77*

*p <0.001.
†p<0.05
‡p <0.01.

the greater the predictability. Considering both sexes together, it appeared that measurement of HDL cholesterol, total cholesterol and triglyceride would be most useful in assessing risk.

The authors advised against the use of HDL/LDL ratios until the physiology and pathophysiology of HDL was better understood. They also pointed out that a good laboratory can achieve a technical error of 5 mg/dl in measuring HDL-C. This error is uncomfortably high when one realizes that the average male HDL-C is 45 mg/dl while a significant increase in CHD risk is present at 35 mg/dl.

Remember that this study applies only to older age groups.

3. Cooperative Lipoprotein Phenotyping Study (17). In this study, the relationship between CHD prevalence and fasting lipid levels was evaluated by a case-control study in 5 populations listed below:

   i. Male civil service employees in Albany, New York.

   ii. General population of black and white men and women in Evans County, Georgia.

   iii. General population of men and women in Framingham, Massachusetts.

   iv. General population of men of Japanese ancestry living in Honolulu.

v. General population of men of Japanese ancestry living in San Francisco.

All CHD cases were contrasted with case controls taken from the same population. Fasting lipid and lipoprotein analyses were made using standard procedures. CHD included documented myocardial infarctions (65%), angina pectoris (48%) and/or coronary insufficiency (7%) established by clinical criteria.

The results of this study were in basic agreement with the two listed previously. The prevalence of CHD decreased as the HDL level rose up to a point but then leveled off at HDL cholesterol levels of 45 mg% or more (Table 5). This leveling off was not observed in the Framingham Study (16).

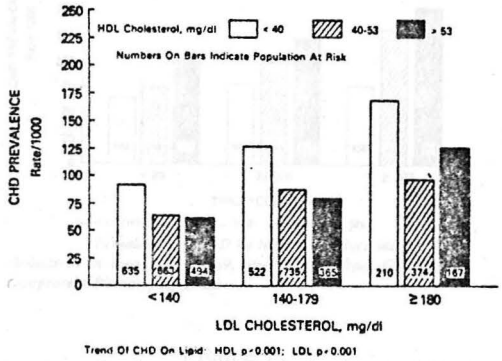
Table 5.

TABLE 5. *Prevalence of CHD by HDL Cholesterol Level, Men aged 50-69*

HDL Cholesterol level	Number		Rate/1000
	CHD	Total population	
All levels	383	4165	92.0
Less 25	9	50	180.0
25-34	78	631	123.6
35-44	133	1406	94.6
45-54	91	1168	77.9
55-64	45	578	77.9
65-74	17	215	79.1
75 plus	10	117	85.5

Using the pooled data, standardized discrimant coefficients for HDL and LDL cholesterol were almost identical in magnitude, indicating similar strong associations with CHD prevalence. No association between triglyceride and CHD prevalence was found.

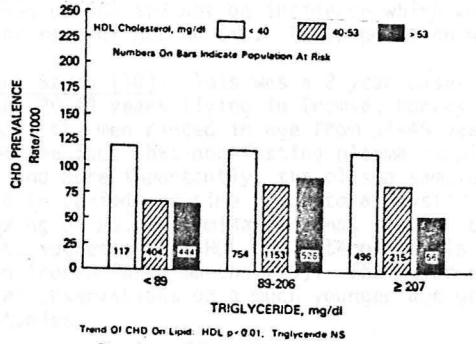
Pairs of lipids were also compared. CHD prevalence increased with increasing LDL levels at each HDL level; at each LDL level, CHD prevalence was greater in individuals with low HDL levels and less in those with moderate or high HDL levels (Fig. 5).



Prevalence of CHD by levels of LDL and HDL cholesterol in men aged 50-69. Pooled data from Cooperative Lipoprotein Phenotyping Studies.

Figure 5.

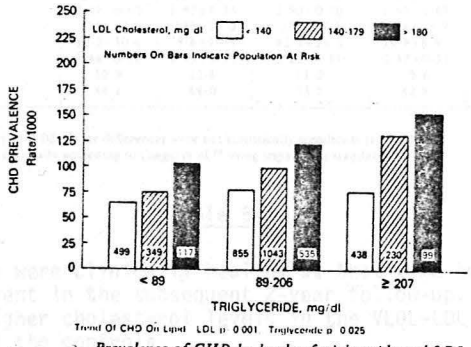
Figure 6 shows similar data for HDL cholesterol and plasma triglyceride. The prevalence for CHD in subjects with low triglyceride levels is similar to that of subjects with intermediate or high triglyceride levels. However, there is a slight inverse association between CHD prevalence and HDL cholesterol level within each of the three triglyceride ranges.



Prevalence of CHD by levels of triglyceride and HDL cholesterol in men aged 50–69: Pooled data from Cooperative Lipoprotein Phenotyping Studies.

Figure 6.

When LDL levels are compared to triglyceride levels (Fig. 7), higher levels of LDL are associated with higher CHD prevalence within each triglyceride range. Also, increasing plasma triglyceride levels are associated with increased CHD prevalance when LDL cholesterol levels are taken into account but this trend is not seen when the HDL level is taken into account (Fig. 6).



Prevalence of CHD by levels of triglyceride and LDL cholesterol in men aged 50–69. Pooled data from Cooperative Lipoprotein Phenotyping Studies.

Figure 7.

The importance of this study was the observation of an inverse relationship between HDL cholesterol and CHD prevalence in several different populations using modern quantitative lipoprotein techniques, suggesting that the inverse HDL-CHD association is characterized by a high degree of generality and strength.

One must remember that this study includes information only on prevalence of prior episodes of CHD and not on incidence which would provide information of the precursor role of HDL on CHD. The population was also older (ages 50-69).

4. The Tromsø Heart Study (18): This was a 2-year case-control follow-up study of 6595 men aged 20-49 years living in Tromsø, Norway. At the time of the analysis in 1974, the men ranged in age from 37-49 years. A major drawback to the study is the fact that non-fasting plasma samples were assayed for plasma lipids, and more importantly, the plasma samples had been frozen at -20°C for variable periods of time prior to analysis. The full impact of long term freezing on HDL determinations has not yet been determined but preliminary data suggests that HDL cholesterol levels actually increase after plasma has been frozen for 6 months (19). The major strength of the study is that it makes observations on a much younger age group as compared to the previous studies.

The results, in brief indicated that plasma HDL cholesterol levels averaged 35% lower in the cases than in the controls (Table 5-a). This difference applied not only to those who had a myocardial infarction before 1974, but

PLASMA-LIPOPROTEIN-LIPID CONCENTRATIONS, BLOOD-PRESSURE, RELATIVE BODY-WEIGHT AND CIGARETTE CONSUMPTION IN CORONARY-HEART-DISEASE CASES AND CONTROLS

—	All cases (N=17)	All controls (N=31)	Cases with previous C.H.D. history (N=6)	Controls (N=11)	Cases without previous C.H.D. history (N=11)	Controls (N=20)
Plasma-H.D.L.-cholesterol (mg/dl)	25·6±6·95*	39·4±8·37	25·2±4·73*	37·2±8·44	25·8±8·11*	40·6±8·29
Plasma-cholesterol density <1.063 (mg/dl)	222±30·8*	190±41·9	219±23·2	190±49·7	224±35·2†	190±38·4
Plasma-total-cholesterol (mg/dl)	248±33·1	229±41·1	244±25·6	227±44·6	250±37·6	231±40·1
Plasma-triglyceride‡ (mmol/l)	2·17±0·92	1·87±1·35	1·90±0·70	1·85±1·48	2·32±1·02	1·87±1·32
Systolic blood-pressure (mm Hg)	128±12·9	129±13·0	124±18·1	126±13·9	130±9·4	130±12·6
Diastolic blood-pressure (mm Hg)	82·2±10·6	79·8±10·4	82·3±14·3	74·9±8·9	82·2±8·7	82·5±10·3
Relative body-weight (g/cm²)	2·44±0·22	2·43±0·31	2·29±0·11	2·37±0·31	2·52±0·24	2·47±0·31
Cigarette consumption (cigs/day)	10·9	11·6	11·2	8·8	10·7	13·1
Age (yr)	44·1	44·0	43·5	42·9	44·4	44·6

Results are expressed as mean±SD.
Cases against controls: * P<0·005; † P<0·02. Other differences were not statistically significant (P>0·05).
‡Plasma-triglyceride was measured manually according to Giegel et al.[18] using tripalmitin standards.

Table 5-a.

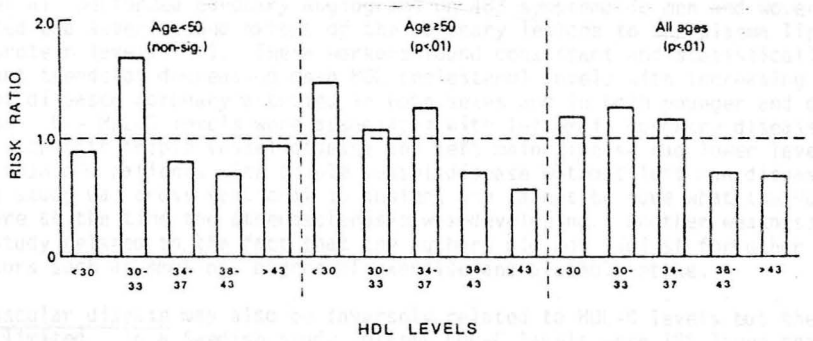
also to those who were clinically healthy at that time but who had their first coronary event in the subsequent 2-year follow-up. The coronary group also had higher cholesterol levels in the VLDL-LDL (d<1.063 gm/ml) fraction than did the controls.

In a recent follow-up to this study, the HDL cholesterol apoA-I levels in serum were measured in 12 subjects who developed myocardial infarcts between 1974 and 1976. Both the HDL-cholesterol and apoA-I levels were lower in the CHD victims than in controls (20) and the HDL-C:apoA-I ratio did not differ between patients and controls, suggesting that HDL composition

did not change markedly in patients with CHD.

5. The Israeli Ischemic Heart Disease Study (21). This prevalence study started in 1963, and followed about 10,000 men aged 40 and over since that time. The participants were civil servants and municipal employees in the areas of Jerusalem, Tel-Aviv, and Haifa and the subjects came from six different regions of birth - Israel, Eastern Europe, Central Europe, Southeastern Europe, The Middle East (excluding Israel), and North Africa. Non-fasting blood specimens were used for analysis and standard lipoprotein technology was employed on fresh specimens.

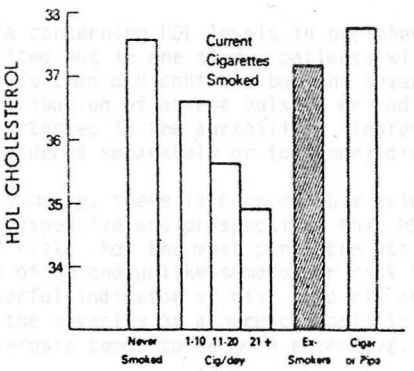
   This study demonstrated that HDL cholesterol levels were inversely related to the incidence of CHD but there are some interesting additions to the information developed by the other studies. First, the risk did not appear until after age 50 years (Fig. 8). Second, cigarette smokers had



Ratio of new MI events in relation to HDL cholesterol. (1.00 is the level for the total population. Ratio = incidence at quintile/overall incidence.)

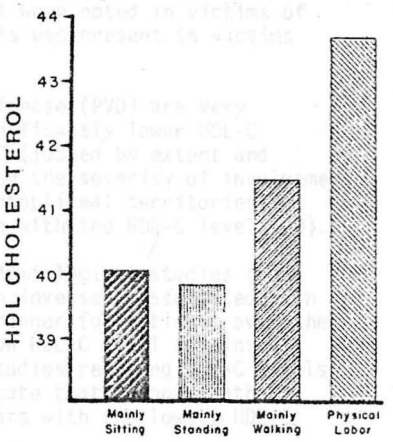
Figure 8.

lower HDL cholesterol levels than did non-smokers or ex-smokers and the effect seemed dose-related. Cigar and pipe smokers had no such change in their HDL levels (Fig. 9). Third, HDL levels were higher in the more physically active participants (Fig. 10).



HDL cholesterol and smoking.

Figure 9.



HDL cholesterol and physical activity on the job (1965 data).

Figure 10.

6. Oslo Heart Study (22): The results of this study are essentially the same as those described above. It suffers from the criticism that frozen serum samples were used for the measurement of HDL and the validity of the HDL measurement on frozen plasma or serum is not known.

In addition to the above population studies, attempts are being made to correlate the degree of atherosclerosis with plasma HDL-C levels in individual patients. Jenkins et al., working in Australia studied the severity of coronary atherosclerosis in 41 patients undergoing coronary angiography and related their findings to plasma lipid levels (23). They noted a strong inverse association of the HDL-C concentration with the extent of coronary atherosclerosis. They also found a direct significant correlation of the extent of coronary atherosclerosis with total plasma cholesterol, LDL-cholesterol and the combined LDL-C plus VLDL-triglyceride but these direct associations were weaker than the inverse association with HDL-C.

A similar but more extensive study from the United States was recently published. Pearson et al. performed coronary angiograms on 483 symptomatic men and women and related the severity and extent of the coronary lesions to the plasma lipid and lipoprotein levels (24). These workers found consistant and statistically significant trends of decreasing mean HDL cholesterol levels with increasing numbers of diseased coronary arteries in both sexes and in both younger and older age groups. Low HDL-C levels were associated with left main coronary disease. Patients with both triple vessel disease and left main disease had lower levels of HDL-C than did patients with triple vessel disease without left man disease. Since the study was cross-sectional in design, one cannot be sure what the HDL-C levels were at the time the atherosclerosis was developing. Another weakness of this study related to the fact that the authors did not adjust for other risk factors such as smoking, degree of exercise and alcohol intake.

Cerebrovascular disease may also be inversely related to HDL-C levels but the data are very limited. In a Swedish study, plasma HDL-C levels were 18% lower than controls in a group of patients (n=61, 38 male, 23 female) younger than age 55 years who survived an attack of ischemic cerebrovascular disease (25, 26). A similar study from Milan observed that plasma HDL-C levels were 19.7% lower than controls in a group of male patients (n=36) with a clinical diagnosis of transient ischemic attack (27). No difference was found in 14 females with TIA's studied at the same time.

In a recent publication from Japan, low HDL-C levels were noted in victims of myocardial infarction but no difference from controls was present in victims of cerebral infarction (28).

Data concerning HDL levels in peripheral vascular disease (PVD) are very limited but in one study, patients with PVD had significantly lower HDL-C levels than did controls but the severity of the PVD (judged by extent and distribution of absent pulses, or radiographically by the severity of involvement of arteries in the aortoiliac, ileofemoral or femoropopliteal territories, considered separately or together) did not correlate with the HDL-C level (29).

In summary, there is considerable evidence from epidemiological studies (both retrospective and prospective) that HDL-C levels are inversely associated with CHD risk. For the most part, the association is stronger for patients over the age of 50 and unlike some other risk factors, the low HDL-C level remains a powerful indicator of risk into old age. Limited studies relating HDL-C levels to the severity of atherosclerosis in patients indicate that coronary atherosclerosis tends to be more extensive in those patients with the lowest HDL-C

levels. Cerebral atherosclerosis may also be associated with low HDL-C levels but the evidence here is less certain and both sexual and national differences in results have been reported.

Limited data in patients with peripheral vascular disease indicates that this disease process, too, may be related to low HDL-C levels.

It is important to remember that no cause-and-effect relationship has been demonstrated between low HDL-cholesterol levels and accelerated atherosclerosis. Nevertheless the association has stimulated investigators to probe into the structure and metabolism of HDL, looking for clues to explain the alleged antiatherogenic potential of HDL. This information is contained in the next sections.

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25) Rössner, S. et al. Dyslipoproteinemia in patients with ischemic cerebrovascular disease. *Atheros.* 30:199-209, 1978.

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III. The Structure of HDL

HDL is the smallest and the most dense of the lipoproteins (Table 6). It possesses α_1 electrophoretic mobility, a feature which lead to its or ginal name of alpha-lipoprotein. Its composition, like that of the other plasma lipoproteins, is complex. About half of the molecule is protein and the other half is lipid (Table 7).

Table 6. Major Plasma Lipoprotein Families*

Family Name	Particle Size Range	Electrophoretic Mobility	Density Range	Svedberg Flotation Units
	Å		gm/ml	
Chylomicrons	750 - 12,000	Origin	D < 0.94	S_f^0 > 400
VLDL	300 - 700	Pre-beta	D = 0.94 - 1.006	S_f^0 20 - 400
IDL**	200 - 600	beta	D = 1.006 - 1.019	S_f^0 12 - 20
LDL	180 - 300	beta	D = 1.019 - 1.063	S_f^0 0 - 12
HDL	50 - 120	alpha	D = 1.063 - 1.21	

* Modified from (30)
** From Ref. (31).

Phospholipid and cholesterol, most of it esterified, are the major lipids while triglyceride is present to only a slight degree. The protein content of HDL is also complex; seven different peptides have been found in the molecule (Table 8). However, 90% of the protein consists of 2 peptides called apoA-I and apoA-II, (the prefix apo- refers to the lipid-free peptide or protein). ApoC and apoE contribute most of the remaining 10%. By inspection of Table 8, one sees that HDL shares several apoproteins in common with other lipoproteins. A-I and A-II, for example, are also found in chylomicrons and VLDL and the same is true for the C and E peptides. The sharing of these common peptides suggests that HDL

has some metabolic relationship with both chylomicrons and VLDL and mounting experimental evidence indicates this to be true. The known characteristics and functions of the different apoproteins are listed in Table 9, but these will not be discussed in any detail today.

Table 7. Composition of Plasma Lipoprotein Families*
Percent of Dry Weight

Lipoprotein Constituent	Chylomicrons	VLDL	IDL**	LDL	HDL
Protein	1 - 2	6 - 10	22	18 - 22	45 - 55
Triglyceride	80 - 95	45 - 65	33	4 - 8	2 - 7
Unesterified cholesterol	1 - 3	4 - 8	10	6 - 8	3 - 5
Esterified cholesterol	2 - 4	16 - 22	16	45 - 50	15 - 20
Phospholipid	3 - 6	15 - 20	18	18 - 24	26 - 32

* Modified from Ref. (30).
** From Ref. (31).

Table 8. Apoprotein Composition of Human Plasma Lipoproteins*

Properties	Chylomicrons	VLDL	LDL	HDL
Major apoproteins	A-I	B	B	A-1
	B	C-I		A-II
	C	C-II		
		C-III		
		E		
Minor apoproteins	A-II	A-I	C	C-I
	E	A-II		C-II
	PRP**	D+		C-III
				D
				E

* Modified from Ref. (32).
** Proline-rich protein.
\+ Also termed "thin-line" protein and apo A-III.

Table 9. Characteristics of Human Plasma Apoproteins*

Apoprotein	Mol. Wt.	Carbohydrate	Function	Site of Synthesis
A-I	28,300	+	LCAT Activation	Intestine, liver
A-II	17,000	±	?	Intestine, liver
B	?	5%	Triglyceride Transport Cholesterol Transport	Liver, intestine
C-I	6,331	0	? LCAT Activation ? Lipase Activation	Liver
C-II	8,837	0	Lipase Activation	Liver
C-III	8,764	+	? Inhibits Lipase	Liver
D	22,100	?	? Activates LCAT	?
E	33,000	?	?	Liver, ? intestine

* Modified from Ref (33).

Up to this point, we have referred to HDL as a single entity of complex composition. However, HDL is often subdivided into 2 (and sometimes more) fractions usually termed HDL_2 and HDL_3. This subdivision came about because a complex peak with a definite shoulder (arrow) was often observed when human HDL was analyzed by analytical ultracentrifugation (Fig. 11) (34).

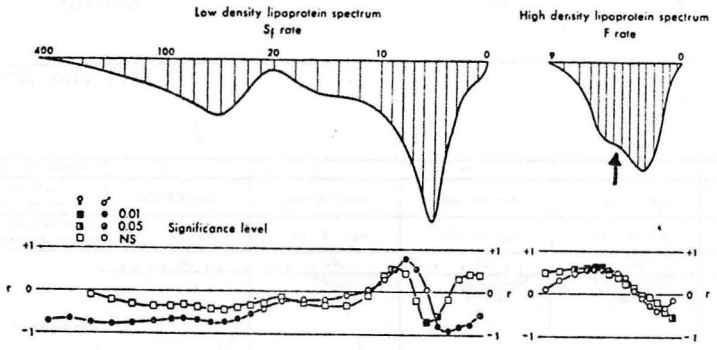


Fig. 11. Correlation observed in males and females between the concentrations of all lipoprotein intervals and $S_f°$ rate of the major S_f 0–12 component.

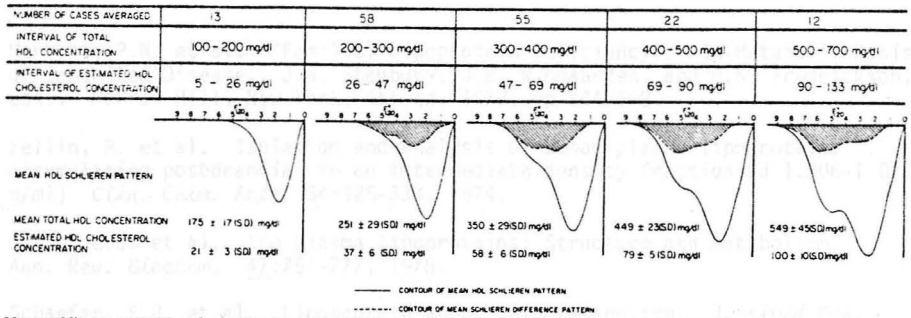
Figure 11.

Some characteristics of these two HDL subgroups are listed in Table 10. Although subdivision of HDL into two fractions appears rather subtle, it may have biological significance. Premenopausal females, for example, have higher HDL_2 concentrations than do males (36,37). However, for any given HDL concentration, the concentrations of these components are similar for men and women (38). As the total HDL

Table 10. Classification and Properties of Human HDL Subfractions*

Property	HDL_2	HDL_3
Electrophoretic Mobility	α	α
Solvent density for isolation	1.063 - 1.125	1.125 - 1.215
Flotation rate, S_f (1.21 gm/ml)	4 - 9	0 - 4
Average hydrated density (gm/ml)	1.094	1.145
Molecular weight	3.9×10^5	1.8×10^5
Diameter (Å)	60 - 140	40 - 100
Composition (% by weight)		
Protein (%)	45	55
Lipid (%)		
Triglyceride	6	7
Cholesterol	43	38
Phospholipid	42	41

* From Ref. (35).

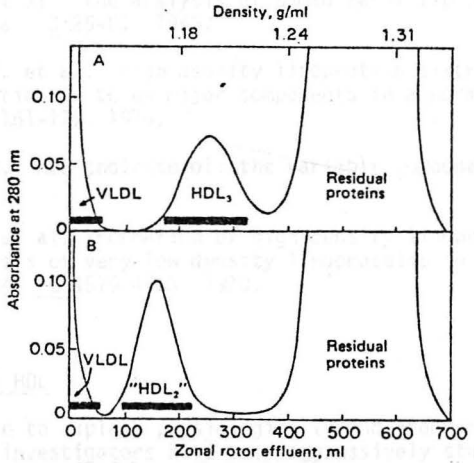


Mean schlieren patterns and associated average H.D.L. cholesterol levels for five centiles of total H.D.L. concentration found in Modesto study.

The shaded area represents the schlieren difference pattern between a given mean pattern and the mean pattern of the next lowest centile of total H.D.L. concentration. For example, the shaded area of the mean schlieren pattern of the 300–400 mg/dl centile represents the schlieren difference pattern obtained by subtracting the 200–300 mg/dl centile pattern from the 300–400 mg/dl centile pattern.

Figure 12.

concentration increases in either sex, it appears that the most significant increase occurs in the HDL_2 fraction (Fig. 12) (39). The HDL_2 fraction is inversely correlated while the HDL_3 fraction is directly correlated with VLDL and LDL levels. The biological significance of HDL_2 and HDL_3 remains uncertain but in vitro experiments indicate that HDL_3 is converted to HDL_2 when VLDL is subjected to lipolysis by lipoprotein lipase (Fig. 13) (40). The results of this experiment plus circumstantial in vivo evidence to be reviewed later suggest that HDL_3 is transformed to HDL_2 during the lipolysis of VLDL triglyceride.



Zonal ultracentrifugal analysis of the incubation mixture for HDL subclasses (*A*) without and (*B*) with lipoprotein lipase. Same experiment as detailed in Fig. 1. A 22.5-ml aliquot of each incubation mixture was centrifuged in a Beckman Ti-14 zonal rotor. Solid blocks indicate fractions that were pooled and then used for further characterization.

Figure 13.

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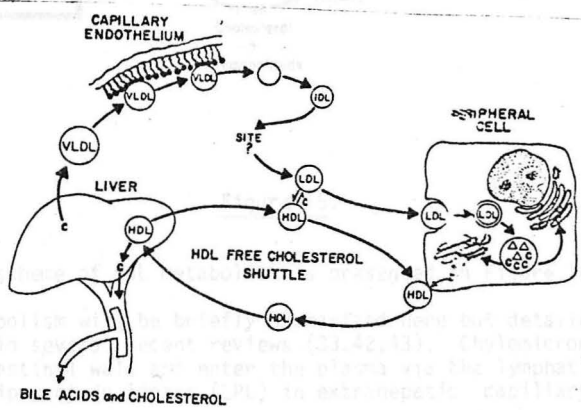
IV. Metabolism of HDL

Prodded by a desire to explain physiologically and biochemically how HDL might be anti-atherogenic, investigators have been aggressively studying the metabolism of HDL in man, experimental animals, perfused organs and tissue culture systems. The working hypotheses regarding the anti-atherogenic effect of HDL are listed below:

1. HDL picks up cholesterol from peripheral tissues and transports it to the liver where it is excreted as cholesterol or bile acids (Fig. 14) (86).



Schematic diagram of lipoprotein and cholesterol metabolism in a man with a bile fistula. C-free (unesterified) cholesterol. (Δ, protein.).

Figure 14.

2. HDL accepts cholesterol from the surface of chylomicrons and VLDL during their catabolism and transports this cholesterol to the liver for excretion.

3. HDL blocks the uptake of LDL in peripheral cells including those responsible for atheroma formation.

We will now review the current facts concerning HDL metabolism to see how well these hypotheses have withstood critical examination.

While significant gaps remain in our knowledge of HDL metabolism, it is clear that HDL metabolism is closely related to that of the other plasma lipoproteins. Thus, the general scheme for lipoprotein metabolism is presented in Figure 15, (41).

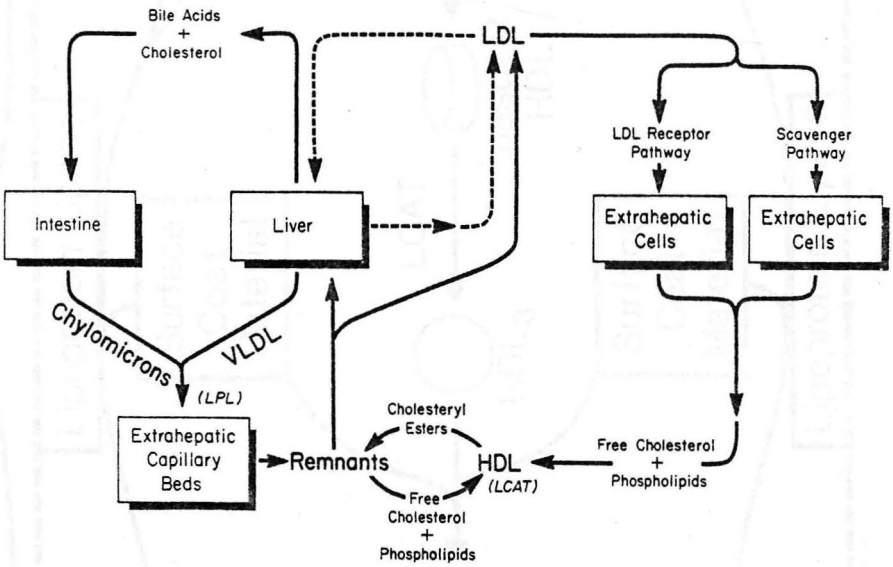


Figure 15.

A more detailed scheme of HDL metabolism is presented in Figure 16.

Lipoprotein metabolism will be briefly summarized here but detailed information can be obtained in several recent reviews (33,42,43). Chylomicrons are synthesized in the intestinal wall and enter the plasma via the lymphatics. They are catabolized by lipoprotein lipase (LPL) in extrahepatic capillary beds and

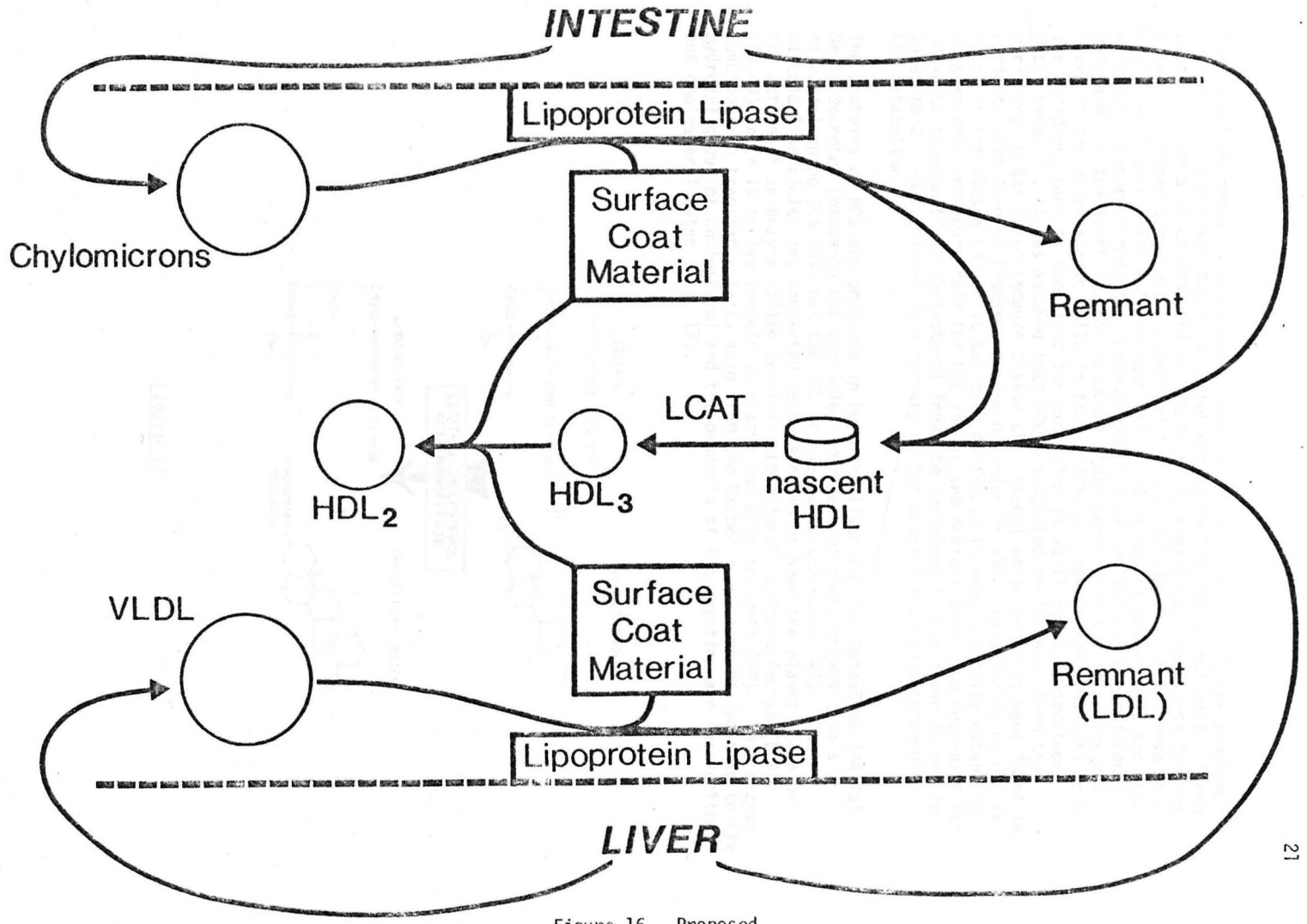


Figure 16. Proposed Scheme for HDL metabolism in Plasma.

the resultant remnants are rapidly cleared by the liver. VLDL are produced primarily in the liver but to a lesser degree in the intestinal wall. These particles are also catabolized by LPL in extrahepatic capillary beds but the catabolic remnants are often converted to LDL. LDL is thought to serve as a cholesterol source for extrahepatic cells and it enters these cells through a specific receptor-mediated process (43). Thus, we believe chylomicrons, VLDL and LDL transport lipids including cholesterol from the intestine and liver to the extrahepatic cells in the periphery. How cholesterol gets from the periphery back to the liver for excretion is still poorly understood. Until recently, it was assumed that HDL transported cholesterol from the periphery to the liver because tissue cholesteryl ester deposits were found in patients with Tangier Disease, a rare disorder in which essentially no HDL is found in the plasma (30). It has been difficult to experimentally establish this reverse transport role for HDL and at the present time, the mechanism for "reverse transport" of cholesterol from the periphery to the liver is poorly understood. What follows is a summary of our present knowledge concerning HDL metabolism.

The proteins of HDL are produced in both the liver and the intestine (44-46). Newly secreted (nascent) HDL particles from each of these organs have a discoidal shape (45,46), but the HDL in plasma is spherical (47). The discoidal particles are converted to spheres in or near the plasma space by the action of an enzyme called lecithin:cholesterol acyltransferase (LCAT) (48,49) which is active largely in plasma but also in lymph (50). This enzyme functions to transfer a fatty acid from the Number 2 position of lecithin to the hydroxyl group of cholesterol and the products of the reaction are lysolecithin and cholesteryl ester (Fig. 17).

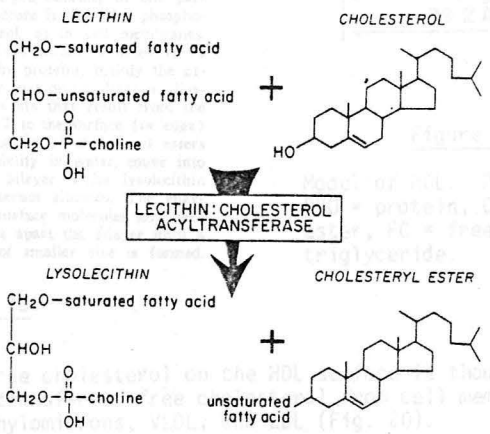
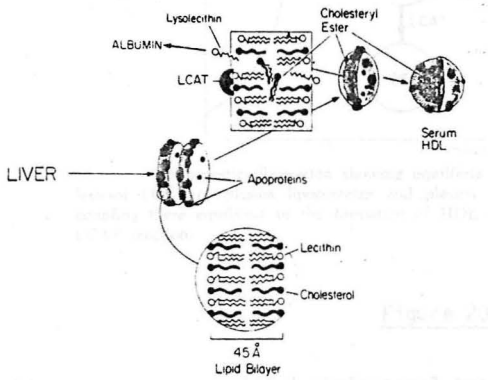


Figure 17.

The net effect of this action is to convert the somewhat polar cholesterol molecule to a non-polar cholesteryl ester and this change in physical properties causes the cholesteryl ester to shift position from the surface of the HDL disc to the non-polar core. As more and more cholesteryl ester shifts to the core, the disc gradually assumes the shape of a sphere with protein, phospholipid and free cholesterol on the surface and cholesteryl ester (plus small amounts of triglyceride) in the non-polar core (Figures 18 and 19).



This schematic diagram depicts the central ideas of our hypothesis of the origin of pseudomicellar HDL of plasma. It suggests that the liver secretes disk-shaped HDL into the blood plasma. The enlarged cutaway of one particle indicates that the basic structure is that of a phospholipid bilayer containing cholesterol, as in cell membranes. The hydrocarbon edge of the disks would of necessity be protected from aqueous plasma by proteins, mainly the arginine-rich apoprotein. The upper part of the diagram illustrates the proposed molecular events that result from the LCAT reaction. Binding of LCAT to the surface (or edge) of the disk is followed by formation of cholesteryl esters which, by virtue of their insolubility in water, move into the hydrocarbon domain of the bilayer. Polar lysolecithin transfers from the surface to serum albumin. The enzymatic transformation consumes surface molecules and generates an oily core which pushes apart the bilayer until a spherical pseudomicellar HDL of smaller size is formed.

Figure 18.

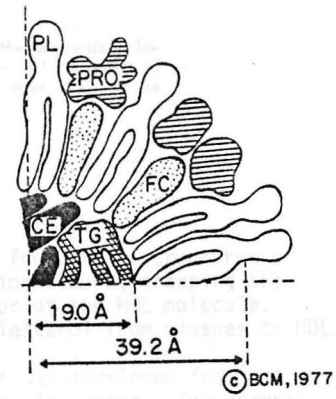
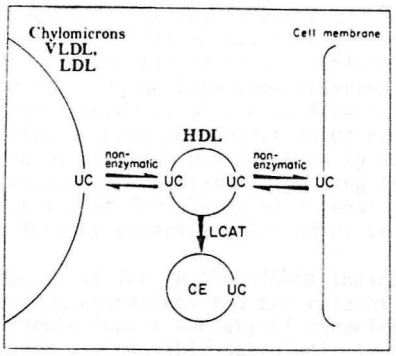


Figure 19.

Model of HDL. PL = phospholipid, PRO = protein, CE = cholesteryl ester, FC = free cholesterol, TG = triglyceride.

The unesterified or free cholesterol on the HDL surface is thought to originate from a non-enzymatic transfer of free cholesterol from cell membranes and also from the surface of chylomicrons, VLDL, and LDL (Fig. 20).



Schematic illustration showing equilibria between the unesterified cholesterol (UC) of plasma lipoproteins and plasma membranes and the effect of coupling these equilibria to the formation of HDL cholesteryl ester (CE) by the LCAT reaction.

Figure 20.

In this scheme, unesterified cholesterol transfers from other structures or macromolecules onto HDL after which the LCAT reaction occurs, trapping the resultant esterified cholesterol within the interior of the HDL molecule. Such a mechanism would achieve net transfer of cholesterol from tissues to HDL.

Most of the scheme for HDL metabolism to this point was developed from rat experiments but some similar findings have been made in humans. For example, HDL apoproteins have been demonstrated by immunochemical methods in biopsies of human small intestine and liver (51). The plasma HDL in patients with LCAT deficiency are disc-shaped (52). Furthermore, patients with cholestasis and alcoholic hepatitis who have very low plasma LCAT activity due to their liver disease have disc-shaped HDL in their plasma (53,54). If the patients with alcoholic hepatitis recover, the disc-shaped HDL reverts to normal spherical forms (54). Very recently, the morphology of HDL from peripheral venous blood was compared to that obtained by catheterization from the hepatic vein of the same patient (55). In this study, the HDL from peripheral venous blood was spherical while that obtained from hepatic venous blood was distinctly disc-shaped. Finally, a negative correlation has been reported between the plasma HDL cholesterol concentration and the length of the resected intestinal segment in patients with Crohn's disease, suggesting that intestinal secretion of HDL occurs in man (56). Thus, the scheme depicted in Figure 18 applies, at least partly, to the human.

Disc-shaped particles do not only arise to novo from the intestine and liver but may also result from the catabolism of chylomicrons and VLDL (57,58). The discs arise when the surface coat of VLDL and chylomicrons becomes redundant

following loss of the apolar triglyceride core during lipolysis. Portions of the redundant surface coat eventually break off as discs which either become HDL particles or else fuse with existing HDL_3 particles to form HDL_2 particles (Fig. 16) (40,59). Irregularities in the surface coat of chylomicrons following exposure to lipoprotein lipase have been observed by scanning electron microscopy (60) suggesting that redundancy of the surface coat actually does occur. Also, in human studies, the intravenous injection of radiolabeled chylomicrons obtained from a chylous pleural effusion was followed by the sequential transfer of radioactive apoA onto HDL particles, suggesting that intestinal chylomicron apoA serves as a precursor for plasma high density lipoprotein apoA (61). These observations lend further experimental support to the scheme proposed in Figure 16.

This scheme for HDL formation in the plasma indicates there is a close relationship between plasma HDL concentrations and the rate of lipolysis of both VLDL and chylomicrons. Evidence from a variety of experiments suggest this relationship does have physiologic and possibly pathologic importance.

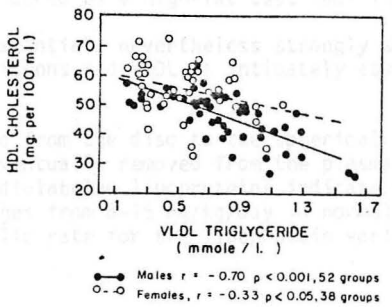
First, it has repeatedly been noted that plasma triglyceride and VLDL levels are inversely related to the plasma HDL concentration (36,62-65) (Table 11, Fig. 21).

—PLASMA LIPID AND LIPOPROTEIN CHOLESTEROL LEVELS IN CONTROLS AND HYPERLIPOPROTEINAEMIC SUBJECTS*

—	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)		
			V.L.D.L.	L.D.L.	H.D.L.
Controls (n=1088)	189±40	87±43	16±11	123±35	50±14
Type I (n=12)	324±197†	3316±2345†	285±199†	22±8†	17±6†
Type II (n=454)	354±91†	135±86	24±19	286±199†	44±12†
Type III (n=66)	441±153†	694±486†	292±158†	111±53	38±19†
Type IV (n=299)	251±64†	438±417†	78±72†	136±42	37±11†
Type V (n=95)	373±190†	2071±2072†	274±213†	72±43†	27±12†

* Mean values ±1 S.D.
† Significantly different ($P<0.01$) from normal as determined by analysis of variance.

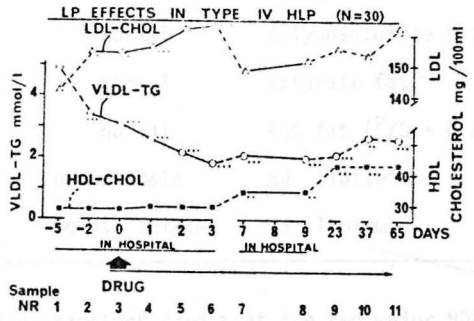
Table 11.



Inverse relationship between HDL cholesterol and VLDL triglyceride (19). Epidemiologic studies have produced conflicting results as to the importance of the concentration of serum VLDL and triglycerides as risk factors for coronary heart disease. Available evidence suggests that the concentration of HDL has an inverse relationship to risk of coronary heart disease. The risk of VLDL and of triglyceride may, therefore, be related to its reciprocal relationship to HDL cholesterol.

Figure 21.

Second, administration of clofibrate, a drug which enhances lipolysis (66) causes an increase in the concentration of HDL in patients with various forms of hyperlipidemia (63,67,68) (Fig. 22).



Mean value for the concentration of VLDL triglycerides, LDL and HDL cholesterol of the Type IV study (from refs. [10—12]). The statistical significance of the changes from sample 1 (*t*-test on individual differences) is given by *** ($P = 0.001$), ** ($P < 0.01$) and * ($P < 0.05$).

Figure 22.

Third, estrogen treatment, which increases triglyceride production (69) and adipose tissue lipoprotein lipase activity (70), also increases concentrations of HDL_2 (71).

Fourth, a highly significant correlation exists between the plasma HDL cholesterol concentration and the fractional removal rate of exogenous triglyceride (Intralipid) (72).

Fifth, plasma LCAT activity is increased during the clearance phase of alimentary lipemia (chylomicronemia) induced by a high-fat test meal in normal subjects (73).

This evidence, while circumstantial, nevertheless strongly suggests that triglyceride metabolism in chylomicrons and VLDL is intimately associated with HDL production.

After HDL has been converted from the disc to the spherical form, it undergoes further metabolism and is eventually removed from the plasma. Studies of HDL turnover in humans using radiolabeled lipoproteins indicate that the synthetic rate for HDL apoprotein ranges from 8-15 mg/kg/day in normal subjects (74-76) while the fractional catabolic rate for the lipoprotein varies from 24 to 31% per day (Table 12).

Table 12. Summary of HDL Turnover Parameters in Normal Human Subjects

Study	Patients	Diet	Fractional Catabolic Rate	Synthetic Rate
			day^{-1}	mg/kg/day
74	normal	polyunsaturate fat	0.28	11.1
74	normal	saturate fat	0.31	15.0
75	normal	40% fat (P/S = 0.2)	0.24	8.5
76	normal male	ad libitum	0.27	12.1
76	normal female	ad libitum	0.25	11.9

Some investigators find that the two major HDL apoproteins (A-I and A-II) disappear from the plasma as a unit (75) but others find that apoA-I is cleared from the plasma more quickly than is apoA-II (77). The reason for the discrepancy is unknown. Thus far, the turnover studies have not provided clues as to why women have higher HDL levels than do men since the HDL turnover characteristics are similar for both sexes (76,77).

Relative to the hypothesis presented in Figure 14, it is instructive to compare the plasma flux of cholesterol on HDL and LDL and the calculations are given in Table 13. While the flux of apoprotein in both LDL and HDL are similar, the cholesterol carried per mg protein is much greater on LDL than on HDL and the total daily flux of cholesterol in LDL is 3 times greater than that in HDL. The point to remember from these calculations is that one should not view cholesterol flux in man simply in terms of LDL transport from the liver to the periphery and HDL transport in the reverse direction since the flux rates for cholesterol on these two lipoproteins are unmatched by several orders of magnitude.

Table 13. Comparison of cholesterol flux on HDL and LDL in normal human subjects.

	LDL (78,79)	HDL
Apoprotein Synthetic Rate (mg/kg/day)	8-14.4	8.5-15
Cholesterol/protein ratio	1.6	0.5
Cholesterol flux (mg/kg/day)	12.8-23	4.3-7.5
Daily cholesterol flux in 70 kg man (mg/day)	896-1610	301-525

The ultimate metabolic fate of spherical HDL particles remains unknown. Following the injection of radiolabeled HDL into rats, radioactivity was found in the liver and kidney (80). However, HDL cholesterol does not appear to be taken up significantly by the rat liver (81,82) and in a recent study comparing the catabolic rate of rat HDL in the perfused rat liver and in the intact animal, it was con-

cluded that the liver catabolized only 7% of the total HDL degraded per day (83). Thus in the rat, it appears that most of the HDL is degraded in non-hepatic tissues. Although similar studies have not been performed in man, evidence has been obtained in human subjects who had catheters placed in their aortas and hepatic veins that LDL cholesteryl ester, but not HDL cholesteryl ester, was taken up by the splanchnic bed, as judged by A-V differences in the concentrations of these two lipoproteins (84). While these experimental results in humans cannot be unambiguously interpreted, they do suggest that LDL, rather than HDL, transports cholesteryl ester to the splanchnic bed (? primarily to the liver).

Another group of investigators, studying patients with T-tubes in their common bile ducts, determined that free cholesterol on HDL appeared much more rapidly in the bile as bile acids or free cholesterol than did free cholesterol on LDL (85,86), and these results were interpreted to indicate that HDL cholesterol might be preferentially taken up and excreted by the liver. It is more likely that these experimenters actually observed exchange rather than net transfer of cholesterol from HDL to bile and the true meaning of their experiments is unknown. Certainly one cannot conclude from the study (85) that HDL cholesterol is preferentially utilized by the liver for secretion into the bile.

In considering the interaction of HDL with tissues, we have already mentioned the proposed non-enzymatic interaction of nascent HDL with cell membranes as depicted in Figure 20. Two other modes of interaction between HDL and tissues have also received experimental attention and these are: (1) the removal of cholesterol from cells by spherical HDL and (2) the interaction of HDL with LDL binding sites on cell membranes.

Spherical HDL, isolated from plasma, has been shown to remove cholesterol from atheromas in vitro while the other lipoproteins were unable to do so (87). Likewise, high density lipoprotein apoproteins mixed with phospholipid were able to remove cholesterol from cultured human skin fibroblasts and rat aortic smooth muscle cells (88,89). The physiologic significance of these observations has not been determined.

Much more attention has been focussed on the possibility that HDL inhibits the binding of LDL to the LDL receptor on cell membranes. This receptor was first described by Dr. Michael Brown and Dr. Joseph Goldstein at our institution and its biological significance was underscored when the impact of its absence was examined in patients with homozygous Familial Hypercholesterolemia (43). The LDL receptor appears necessary for the proper regulation of both intracellular cholesterol metabolism and plasma LDL metabolism (43,79) and in its absence, accelerated atherosclerosis results. A schematic diagram of the LDL receptor pathway is presented in Figure 23 (43). It is important to remember that patients with Familial Hypercholesterolemia lack the normal number of functioning LDL receptors and also have extensive atherosclerosis. Thus, although the LDL receptor serves to deliver cholesterol in the form of LDL into cells, this process does not appear to produce atherosclerosis. The presence of normal functioning LDL receptors and the LDL pathway in cells is therefore thought to represent a system whereby tissues can take up cholesterol without atheroma formation. It is important to stress this point in view of the hypothesis that HDL protects against atherosclerosis by inhibiting the binding of LDL to its receptor because the hypothesis does not make much sense if LDL uptake by the receptor is not actually harmful. With this background information in mind, we will briefly review the data which resulted in this hypothesis.

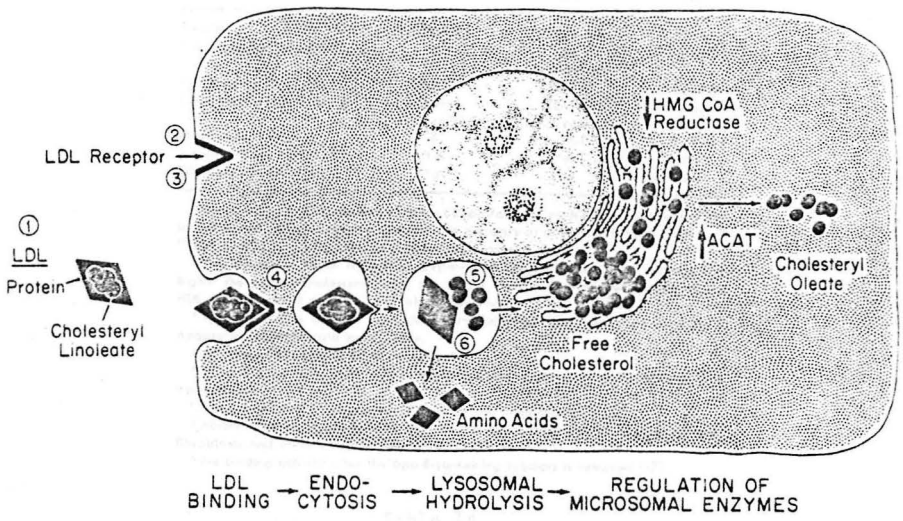


Figure 23.

Carew et al. noted that HDL partially inhibited the uptake and degradation of LDL by cultured porcine arterial smooth muscle cells and also partially suppressed the net increment in cell sterol content induced by LDL (90). These investigators subsequently extended their observations to cultured human fibroblasts (91). Since this observation could not be reproduced in other laboratories (92), the hypothesis was viewed with skepticism. Recent experiments by Mahley and co-workers appear to have cleared up the confusion. These investigators discovered an unusual apoprotein complex in both VLDL and the HDL_2 density region of normal human plasma (1.063-1.125). The complex consists of apoE and A-II linked together by a disulfide bond and the complex was found as a major apoprotein constituent in a minor subclass of normal plasma HDL termed HDL_1 or HDL_C (Table 14) (93,100). HDL_1 was shown to account for most, if not all, of the high affinity binding of HDL (d=1.063-1.215) to LDL receptors on the surface of human fibroblasts (94). Furthermore, the binding of this HDL_1 material is due to the presence of apoE in the molecule (94). The biological significance of this apoE-containing HDL subfraction is unknown but its concentration is increased in human subjects fed 4-6 eggs/day for 4 weeks or 3 eggs/day for 18 weeks (95). This increase in apoE-containing HDL occurs even if the total plasma cholesterol level is unaffected by the consumption of eggs. We do know that this apoE-containing HDL_1 fraction precipitates in the presence of heparin-manganese solution so it is not measured as HDL in the clinical laboratory (vida infra). Therefore, its

Characteristics of LDL, HDL_c, and "typical" HDL[a]

	LDL	HDL_c	"Typical" HDL
Density	1.02–1.06	1.03–1.10[b]	1.06–1.21
Electrophoretic Mobility	β	α_2 or slow α_1[c]	α_1
Particle Size (Å)	160–240	130–250	80–100
Chemical Composition[d]	CE-rich (CE core)	CE-rich (CE core)	Protein, PL
Major Apoproteins	B	E,A-I(A-II)[e]	A-I(A-II)[e]
Heparin Precipitable	Yes	Yes	No[f]
LDL Receptor Binding[g]	Yes	Yes	No[h]

[a] For precise characterization of these lipoproteins for each species see references in text to the particular animal (generalized summary of properties). For definition of "typical" HDL see Summary and Conclusions section, p. 26.

[b] The density at which HDL_c float depends on the cholesteryl ester content. The higher the plasma cholesterol level, the higher the cholesteryl ester content of the HDL_c and the lower the density at which they float.

[c] As the particles become more cholesteryl ester-rich and contain more of the E apoprotein, they migrate more slowly.

[d] CE, cholesteryl esters; PL, phospholipid.

[e] The A-II apoprotein is not a prominent constituent in some species. E, arginine-rich apoprotein.

[f] Not precipitable at low levels of heparin and manganese.

[g] Ability of these lipoproteins to bind to the high affinity cell surface receptor of fibroblasts and smooth muscle cells grown in culture.

[h] No binding activity after the apo-E-containing subclass is removed (47).

Table 14.

presence in the blood bears no relationship to the inverse correlation of HDL-C levels with coronary heart disease. In addition, it is unlikely that HDL_1 is metabolized by the LDL receptor since it does not accumulate in the plasma of patients with receptor-negative homozygous Familial Hypercholesterolemia.

In summary, HDL plays an important role in the catabolism of other lipoproteins but its putative role in reverse cholesterol transport remains unclear. The interaction of HDL with the LDL receptor is due to the presence of an apoE-containing HDL_1 fraction of unknown biological significance and since this fraction is not measured in the clinical laboratory as part of HDL-cholesterol, its presence in plasma does not affect the inverse association of HDL-C with atherosclerosis.

Some consideration should be given to the possibility that cholesteryl esters are transported in plasma by a process similar to that for free fatty acids (FFA). FFA are transported through plasma bound to albumin and although plasma FFA concentrations are normally very low, they exhibit rapid turnover and therefore contribute significantly to energy flux (96). Recently a cholesteryl ester transfer protein (or exchange protein) has been isolated from human plasma (97-99). While it has been shown to facilitate the transfer of cholesteryl ester from HDL to VLDL or LDL, the possibility that it also transfers cholesteryl esters from tissues to lipoproteins or to other tissues has not been explored but studies in this area will no doubt proceed rapidly.

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V. Measurement of HDL

Since HDL is a complex molecule, there are several parameters one can measure to arrive at an HDL concentration. The most common method is to quantify the cholesterol in HDL after the lipoprotein has been separated from the other plasma lipoproteins by precipitation or preparative ultracentrifugation. Newer methods, still largely experimental, involve the radioimmunoassay of the HDL apoproteins, A-I and A-II. If one is interested in HDL_2 and HDL_3 measurements, current methodology is specialized and expensive since analytical ultracentrifugation or zonal ultracentrifugation is required. The estimation of HDL concentrations by lipoprotein electrophoresis and densitometric scanning is not recommended for risk factor analysis.

The precipitation of the non-HDL, apoB-containing lipoproteins from serum remains the most practical way to quantify HDL cholesterol. The precipitation is usually achieved with a mixture of sulfated polysaccharides (Heparin or Dextran sulfate) and divalent cations (Ca^{++}, Mg^{++} or Mn^{++}). Occasionally phosphotungstate has been employed. The general outline of the raction is illustrated below (Fig. 24).

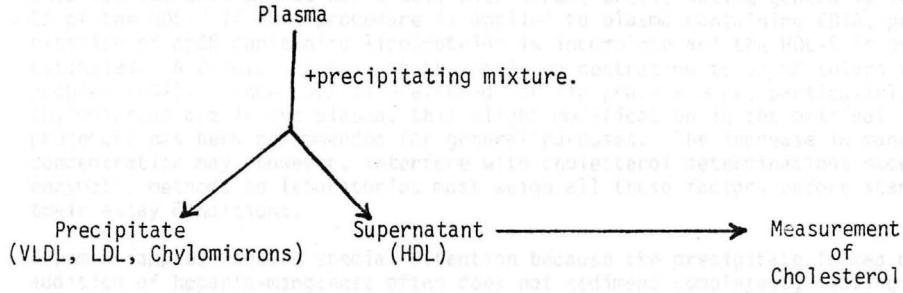


Figure 24. General Scheme for HDL-Cholesterol Quantification using the precipitation technique.

The various procedures used in the HDL-C determination have been extensively evaluated (101-104) and a comparison is given in Table 15 (104).

Comparison of Mean HDL Cholesterol by Several Techniques[a]

Samples							
		Corrected	Heparin-Mn^{2+}		Dextran		
Source	n	d>1.063 fraction[b]	.046 M[c]	.092 M[d]	Sulphate[e] 500 Mg^{2+}	Phosphotungstate[f] Mg^{2+}	Heparin[g] Ca^{2+}
Plasma							
Women	30	61.7	63.9	60.7	58.1	57.4	66.7
Men	27	44.4	45.7	44.1	43.6	42.8	51.5
Children	8	52.2	53.0	50.8	48.0	49.0	57.2
All Subjects	65	53.4	55.0	52.6	50.9	50.3	59.2
Serum							
Women	10	70.0	70.5	66.9	62.4	64.7	75.0
Men	10	44.7	44.2	42.8	40.0	41.6	53.1
All Subjects	20	57.4	57.4	54.8	51.2	53.2	64.0

[a]Results expressed in mg/dl

[b]Corrected for losses of cholesterol during ultracentrifugation and the presence of apoB-associated cholesterol.

[c]According to the Lipid Research Clinics Procedure (12).

[d]According to Warnick and Albers (14).

[e]According to Kostner (17).

[f]According to Lopes et al. (18).

[g]According to Srinivanson et al. (19).

Table 15.

The most widely used procedure is the Heparin-Mn^{2+} method using 46mM maganese chloride and this method works well with serum, precipitating generally less than 2% of the HDL. If this procedure is applied to plasma containing EDTA, precipitation of apoB containing lipoproteins is incomplete and the HDL-C is overestimated. A 2-fold increase in the $MnCl_2$ concentration to 92 mM solves the problem (104). Since EDTA is preferred for lipoprotein work, particularly when chylomicrons are in the plasma, this slight modification in the original procedure has been recommended for general purposes. The increase in manganese concentration may, however, interfere with cholesterol determinations made with enzymatic methods so laboratories must weigh all these factors before standardizing their assay conditions.

Lipemic samples require special attention because the precipitate formed upon addition of heparin-manganese often does not sediment completely, leaving a turbid supernatant. The simpliest way around this problem is to filter the turbid supernatants with a 0.22 μm filter after which cholesterol determinations can be made on the clear filtrates (105).

Since cholesterol makes up only 15-20% of the weight of HDL while protein makes up 50%, efforts have been made to quantify the HDL protein for use as another index of the plasma concentrations of this lipoprotein. Various immunoassay procedures have been developed to quantify apoA-I and apoA-II, and most of the work in this area has come from laboratories in Seattle and St. Louis (104,106, 107). While apoA-I and apoA-II assays do not have immediate clinical relevance, it is possible, and likely, that they will be used in the future. For that reason, some representative studies are summarized below.

Representative data on HDL apoprotein assays from Seattle is given in tables 16 to 18 (104). The data in Table 16 indicates that apoA-II levels increase slightly with age in women but not in men.

Plasma A-II in Normal Subjects
(mean ± S.D., mg/dl)

Age	Number		Plasma A-II	
	Men	Women	Men	Women
20-29	27	82	34 ± 4	35 ± 6
30-39	53	33	33 ± 5	35 ± 7
40-49	55	44	34 ± 5	37 ± 5
50-59	35	27	33 ± 5	38 ± 6

Table 16.

This data in Table 17 indicate that apoA-I levels increase significantly with age, especially in women. Considered together, the data in these two tables indicate that HDL apoA-I/apoA-II ratios change somewhat with increasing age.

Plasma A-I Levels in Normal Subjects
(mean ± S.D., mg/dl)

Age	Number		Plasma A-I	
	Men	Women	Men	Women
20-29	50	114	117 ± 18	132 ± 26
30-39	77	39	117 ± 19	135 ± 26
40-49	77	62	120 ± 10	137 ± 22
50-59	55	37	125 ± 22	140 ± 32
60-65	4	5	126 ± 20	168 ± 23

Table 17.

Table 18 presents some pooled data from the Seattle lab. It is clear that both HDL-C and apoA-I are increased in women as compared to men. Women taking estrogen preparations also show increased HDL-C and apoA-I levels as compared to controls. Estrogen-progesterone combinations, however, do not appear to have as marked an effect on HDL-C and A-I levels.

Plasma HDL-Cholesterol, A-I and A-II Levels in Normal Subjects[a]

Subject group	n	HDL CH	A-I	A-II
All men	192	45	120	33
All women	188	55	135	36
Women taking no estrogen[b]	92	54	130	34
Women on estrogen	19	61	149	39
Women on estrogen and progesterone	56	54	140	39

[a]Normal subjects refers to a subset of an industrial population who were selected independently of their lipid levels. Results expressed as mean levels in mg/dl.
[b]Refers to a subset of women from the population who had taken no medication for 2 weeks before blood drawing.

Table 18.

In St. Louis, Dr. Schonfeld and co-workers have been collecting data on hyperlipidemic individuals classified by lipoprotein phenotyping because they were unable to carry out the family studies needed to establish genetic diagnoses (107).

These workers noted that HDL cholesterol levels were lowest in patients with hypertriglyceridemia whereas HDL triglyceride levels were highest in these patients (Table 19, A and B). However, when they measured the A-I and A-II

A

Lipoprotein Lipid Levels in Hyperlipidemia (Men)[a]

Phenotype	TG	LDL-C	HDL-C	HDL-TC
N (41)	90 ± 34	121 ± 33	47 ± 12	9 ± 5
IIa (10)	141 ± 30	240 ± 49[b]	36 ± 7[c]	10 ± 5
IIb (10)	258 ± 111[b]	242 ± 69[b]	37 ± 8[c]	13 ± 4[c]
III (6)	223 ± 59[b]	126 ± 27	42 ± 6	18 ± 4[b]
IV (14)	393 ± 130[b]	123 ± 29	34 ± 15[b]	17 ± 6[b]
V	2310 ± 884[b]	57 ± 19[b]	21 ± 5	26 ± 6[b]

[a]Results are in mg/dl; mean ± 1 S.D.; TG = triglyceride; C = cholesterol; LDL = low density lipoproteins; and HDL = high density lipoproteins. Type II patients had LDL-C > 190 mg/dl. Types III were diagnosed by VLDL-C/VLDL-TG > 0.42 and isoelectric focusing of VLDL apoprotein. Types IV and V had TG > 250 mg/dl. Means of patient groups are compared to the mean of normolipidemic controls (N). Numbers of individuals per group are in parenthises. (The same designations pertain in Tables II, IV-VIII).
[b]$p<0.0001$.
[c]$p<0.005$.

B

Lipoprotein Lipid Levels in Hyperlipidemia (Women)[a]

Phenotype	TG	LDL-C	HDL-C	HDL-TG
N (33)	74 ± 32	117 ± 29	54 ± 15	10 ± 5
IIa (12)	136[b] ± 31	230[b] ± 35	50 ± 10	13 ± 5
IIb (9)	243[b] ± 40	225[b] ± 58	44[c] ± 15	17[b] ± 4
III (5)	505[d] ± 456	123 ± 26	45[d] ± 11	17[d] ± 6
IV (5)	430[d] ± 204	105 ± 45	30[b] ±5	23[b] ± 8
V	1419	81	24	22

[a]Results are in mg/dl; mean ± 1 S.D.; TG = triglyceride; C = cholesterol; LDL = low density lipoproteins; and HDL = high density lipoproteins. Type II patients had LDL-C > 190 mg/dl. Types III were diagnosed by VLDL-C/VLDL-TG > 0.42 and isoelectric focusing of VLDL apoprotein. Types IV and V had TG > 250 mg/dl. Means of patient groups are compared to the mean of normolipidemic controls (N). Numbers of individuals per group are in parenthises. (The same designations pertain in Tables II, IV-VIII).
[b]$p<0.0001$.
[c]$p<0.05$.
[d]$p<0.01$.

Table 19.

levels in these patients, only those with the highest triglyceride levels (type V phenotype) showed any significant reduction in A-I and A-II levels (Table 20, A and B).

A

Apolipoprotein A-I and A-II Levels in Hyperlipidemia (Men)[a]

Phenotype	ApoA-I	ApoA-II
N (19)	109 ± 24	40 ± 8
IIa (8)	108 ± 37	38 ± 10
IIb (13)	124 ± 44	44 ± 7
III (6)	121 ± 26	41 ± 9
IV (17)	104 ± 30	36 ± 8
V	90[b] ± 14	30[b] ± 12

[a]Results are in mg/dl given as means ± 1 S.D.; nonpaired t-tests are calculated using log transformed data.
[b]$p<0.05$.

B

Apolipoprotein A-I and A-II Levels in Hyperlipidemia (Women)[a]

Phenotype	ApoA-I	Apo-A-II
	mg/dl	
N (21)	122 ± 27	37 ± 6
IIa (11)	111 ± 19	45[b] ± 10
IIb (8)	109 ± 24	45 ± 13
III (5)	102 ± 14	43 ± 12
IV (5)	96[c] ± 18	38 ± 14
V (1)	81	44

[a]Results are in mg/dl given as means ± 1 S.D.; nonpaired t-tests are calculated using log transformed data.
[b]$p<0.03$.
[c]$p<0.01$.

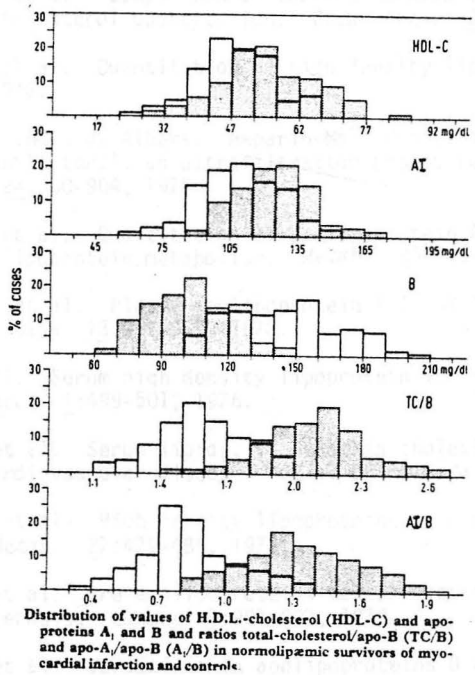
Table 20.

These findings indicate that the apparent inverse correlation of HDL-cholesterol concentrations with plasma triglyceride levels may actually reflect a change in HDL composition more than a change in the number of molecules of HDL present in plasma in all but the most severe forms of hypertriglyceridemia. Thus, hypertriglyceridemia is associated with a shift in HDL lipid content such that each molecule contains more triglyceride and less cholesterol. Measurement of HDL cholesterol in this setting, therefore, may not reflect a change in HDL particle concentration. The significance of these changes is now under study.

Studies relating A-I and A-II levels to the risk for atherosclerosis are already being published. There is uniform agreement that patients who survive a myocardial infarction have lower A-I levels than controls and this change parallels that for HDL-C (108-110). Similar results were found in patients with peripheral

vascular disease (109).

In a recent study comparing lipid, lipoprotein, and apoprotein levels in 218 survivors of myocardial infarction with 160 controls, the investigators found the expected lower HDL-C, lower A-I, higher total cholesterol, and higher apoB concentrations observed in previous studies and there was considerable overlap between the infarction victims and the controls (Fig. 25) (111).



Distribution of values of H.D.L.-cholesterol (HDL-C) and apoproteins A_I and B and ratios total-cholesterol/apo-B (TC/B) and apo-A_I/apo-B (A_I/B) in normolipæmic survivors of myocardial infarction and controls.

Figure 25. Clear blocks represent controls and shaded areas represent the survivors of myocardial infarcts.

When the ratio of total cholesterol to apoB or the ratio of A-I/B were plotted, however, a bimodal distribution results and reduces the overlap between the MI victims and the controls (Fig. 25). Whether this type of analysis will have any predictive value with regard to cardiovascular risk is not yet known.

The same group of investigators has also determined that A-I levels drop immediately following a myocardial infarction while HDL cholesterol levels remain unchanged (112). After 25-30 days, the A-I levels return to baseline.

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VI. Factors Influencing Plasma HDL-cholesterol levels.

A variety of factors including age, sex, time in the menstrual cycle, body weight, seasons of the year, exercise, cigarette smoking, alcohol consumption, diet, and genetic factors are believed to influence HDL levels. These findings are briefly summarized below:

1. The influence of age and sex in HDL-cholesterol levels in the United States. The most extensive data has been generated in a prevalence study conducted in the Lipid Research Clinics Program. The data is presented in tabular form for each sex (Tables 21 and 22) and is depicted for both sexes in Figure 26 (113).

Table 21.

Mean Plasma Total Cholesterol, VLDL-C, LDL-C and HDL-C in White Females in 11 North American Populations[a]

AGE	N	Total cholesterol[b] mg/dl	VLDL-C[b] mg/dl	LDL-C[b] mg/dl	HDL-C[b] mg/dl
5-9	126	164.0 ± 1.8	9.7 ± 0.7	100.4 ± 2.1	53.2 ± 1.0
10-14	248	160.1 ± 1.5	10.9 ± 0.4	97.4 ± 1.3	52.2 ± 0.7
15-19	297	159.5 ± 1.6	11.8 ± 0.5	95.7 ± 1.5	52.3 ± 0.7
20-24	199	170.3 ± 2.5	13.5 ± 0.6	103.7 ± 2.2	53.3 ± 1.0
25-29	314	179.5 ± 1.7	13.4 ± 0.5	110.2 ± 1.6	56.0 ± 0.8
30-34	337	179.2 ± 1.7	12.2 ± 0.5	111.3 ± 1.5	56.0 ± 0.7
35-39	300	189.6 ± 2.1	15.4 ± 0.7	119.7 ± 2.0	55.0 ± 0.8
40-44	319	197.5 ± 1.9	14.7 ± 0.5	125.1 ± 1.8	57.8 ± 0.9
45-49	329	206.2 ± 2.0	17.4 ± 0.7	129.4 ± 1.9	59.4 ± 1.0
50-54	257	217.3 ± 2.4	17.2 ± 0.7	138.1 ± 2.3	62.0 ± 1.0
55-59	251	228.7 ± 2.4	20.7 ± 1.0	146.1 ± 2.4	62.2 ± 1.1
60-64	145	232.3 ± 3.7	16.7 ± 1.8	152.0 ± 3.6	63.8 ± 1.4
65-69	130	234.1 ± 4.0	17.0 ± 1.3	153.8 ± 4.1	63.3 ± 1.8
70+	143	224.5 ± 2.8	15.6 ± 1.2	148.6 ± 2.7	60.7 ± 1.4

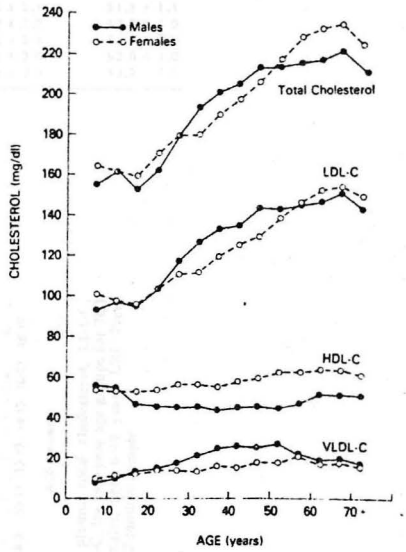
[a]Source: LRC Prevalence Study, Visit 2 Random Sample.
[b]Mean ± SEM.

Mean Plasma Total Cholesterol, VLDL-C, LDL-C and HDL-C in White Males in 11 North American Populations[a]

Age	N	Total cholesterol[b] mg/dl	VLDL-C[b] mg/dl	LDL-C[b] mg/dl	HDL-C[b] mg/dl
5-9	148	155.3 ±'1.8	8.2 ± 0.5	92.5 ± 1.8	55.8 ± 1.0
10-14	299	160.9 ± 1.5	9.9 ± 0.4	96.8 ± 1.4	54.9 ± 0.7
15-19	299	153.1 ± 1.4	12.8 ± 0.5	94.4 ± 1.3	46.1 ± 0.6
20-24	118	162.2 ± 2.5	13.7 ± 0.8	103.3 ± 2.4	45.4 ± 1.0
25-29	253	178.7 ± 2.1	17.4 ± 0.9	116.7 ± 1.9	44.7 ± 0.7
30-34	403	193.1 ± 1.8	21.3 ± 0.9	126.4 ± 1.6	45.5 ± 0.6
35-39	372	200.6 ± 1.9	24.1 ± 1.0	133.2 ± 1.7	43.5 ± 0.6
40-44	385	205.2 ± 1.9	25.5 ± 1.2	135.6 ± 1.6	44.2 ± 0.6
45-49	327	213.4 ± 1.9	24.4 ± 1.1	143.7 ± 1.8	45.5 ± 0.6
50-54	340	213.2 ± 1.9	26.8 ± 1.1	142.3 ± 1.7	44.1 ± 0.6
55-59	261	215.0 ± 2.2	21.6 ± 1.1	145.8 ± 2.1	47.6 ± 0.9
60-64	131	216.6 ± 3.3	18.9 ± 1.3	146.3 ± 3.1	51.5 ± 1.3
65-69	105	221.0 ± 3.8	19.7 ± 2.0	150.4 ± 3.5	51.1 ± 1.5
70 +	119	210.3 ± 3.4	17.0 ± 1.2	142.9 ± 2.9	50.5 ± 1.7

[a]Source: LRC Prevalence Study, Visit 2 Random Sample.
[b]Mean ± SEM.

Table 22.



Mean plasma total cholesterol, LDL-C, HDL-C and VLDL-C by 10-year age groups for 3581 males and 3426 females. LRC Prevalence Study, Visit 2 random sample.

Figure 26.

In females, a mean HDL-C level of about 53 mg/dl is maintained between ages 5-24 years after which it rises to 56-58 mg/dl between 25-44 and to a peak of 64 mg/dl in the years 60-65. It then drops to 61 mg/dl by age 70 and beyond.

In males, a mean HDL-C of 55 mg/dl is observed from 5-14 years but after 14, the level drops to 46 mg/dl and remains at 44-46 mg/dl through the 50-54 age range, after which it rises to about 51 mg/dl.

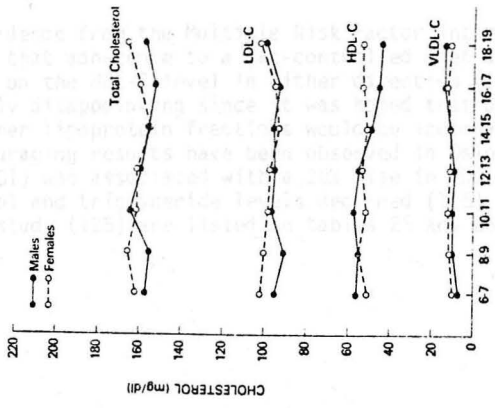
Values for persons 6-19 years of age are presented in Table 23 and Figure 27 (113). Between ages 6 and 13 years, males have slightly higher HDL-C levels than females but after age 13, females have substantially higher levels (113). Similar observations for children have been reported in two other large scale studies from Bogalusa and Cincinnati (114,115,116).

Mean Plasma Total Cholesterol, VLDL-C, LDL-C, and HDL-C, in White Children Ages 6-19 years, in Selected North American Populations [a]

Age	N	Total cholesterol[b] mg/dl	VLDL-C[b] mg/dl	LDL-C[b] mg/dl	HDL-C[b] mg/dl
Males					
6-7	67	155.9 ± 2.5	6.9 ± 0.6	95.0 ± 2.5	56.0 ± 1.3
8-9	71	155.6 ± 2.6	9.6 ± 0.8	90.5 ± 2.4	55.6 ± 1.5
10-11	93	164.0 ± 2.9	10.4 ± 0.8	96.8 ± 2.8	57.3 ± 1.2
12-13	742	159.5 ± 1.8	9.0 ± 0.5	95.4 ± 1.7	55.9 ± 1.1
14-15	129	154.8 ± 2.3	10.7 ± 0.7	95.5 ± 2.1	49.2 ± 1.0
16-17	160	152.1 ± 1.8	13.5 ± 0.7	93.2 ± 1.6	45.6 ± 0.8
18-19	67	156.6 ± 3.3	13.8 ± 0.9	99.3 ± 3.2	43.7 ± 1.0
Females					
6-7	58	162.2 ± 2.4	9.8 ± 1.1	100.5 ± 3.3	50.1 ± 1.7
8-9	60	166.1 ± 2.7	10.6 ± 1.1	100.0 ± 2.9	55.7 ± 1.4
10-11	101	161.3 ± 2.3	11.8 ± 0.8	98.1 ± 2.1	51.5 ± 1.1
12-13	102	160.8 ± 2.3	10.6 ± 0.7	97.7 ± 2.0	53.0 ± 1.0
14-15	122	154.9 ± 2.4	10.6 ± 0.6	93.5 ± 2.1	51.0 ± 1.0
16-17	164	159.7 ± 2.1	12.3 ± 0.6	95.2 ± 2.0	52.8 ± 1.0
18-19	53	165.7 ± 4.1	11.1 ± 1.1	101.8 ± 3.5	53.2 ± 1.6

[a]Source: LRC Prevalence Study, Visit 2 Random Sample.
[b]Mean ± SEM.

Table 23.



Mean plasma total cholesterol, LDL-C, HDL-C, and VLDL-C, by two-year age groups for 763 males and 676 females, ages 6-19 years. LRC Prevalence Study, Visit 2 random sample.

Figure 27.

The Bogalusa study has actually made measurements on children since birth and the results indicate that the plasma HDL cholesterol levels (α) rise steadily from birth through the age of 5 years (Table 24) (116).

Serum Lipoprotein Cholesterol and Triglycerides by Age
Bogalusa Heart Study, 1973-1974. Mean (95th Percentile)

Age	N[a]	Cholesterol				Triglycerides[b]
		Total	α-[b]	β-[b]	Pre-β[b]	
Birth	419	70 (103)	36 (60)	30 (50)	4 (12)	40 (54)
6 Months	312	135 (185)	51 (88)	74 (111)	10 (25)	92 (169)
1 Year	291	145 (193)	53 (81)	83 (121)	9 (25)	82 (158)
2½-5½ Years	694	157 (198)	60 (90)	91 (129)	6 (18)	63 (113)
5-14 Years	3446	165 (215)	68 (104)	89 (130)	8 (22)	69 (126)

[a]Nonfasting included in total numbers; numbers vary slightly for each variable.
[b]Fasting samples only.

Table 24.

2. Obesity and HDL-Cholesterol Levels. HDL cholesterol levels are inversely correlated with body weight both in adults (16,21,117,118,120,121) and in children aged 10-14 (116). This finding is not totally unexpected since obesity is positively associated with plasma triglyceride levels and plasma triglyceride levels are inversely associated with HDL-C concentrations. It is reasonable to suppose that weight reduction might be associated with a rise in HDL-C levels 63,121) but this change has not been uniformly observed (118,119). Studies in this area are limited, however, and it is premature to predict how weight loss will affect HDL-C levels in human subjects.

3. Diet and HDL-Cholesterol Levels: Dietary factors influence HDL-cholesterol levels. The response of HDL to the low fat, low cholesterol diet used to treat hyperlipidemia is of major concern and data is available from several sources (121-125). Wilson et al. found that the Fat-controlled American Heart Association (AHA) diet unrestricted in carbohydrate content lowered all plasma lipoproteins levels but specifically, the HDL level was lowered an average of 6.2 ± 2.5% (note: quantification was by lipoprotein electrophoresis) (122). In contrast, if this diet was modified such that 30% of the calories were derived from carbohydrate and 50% from fat ($^{P}/_{S}$ = 1.5), then HDL levels did not change while VLDL and LDL levels dropped (123).

Recent evidence from the Multiple Risk Factor Intervention Trial (MRFIT) indicates that adherence to a fat-controlled diet does not have a major influence on the HDL-C level in either direction (121,124). This observation is actually disappointing since it was hoped that dietary-induced reduction in the other lipoprotein fractions would be accompanied by increases in HDL-C. More encouraging results have been observed in Oslo where a 25% fat diet ($^{P}/_{S}$ = 1.01) was associated with a 20% rise in HDL-C levels while total cholesterol and triglyceride levels declined (125). Data from the MRFIT (124) and Olso study (125) are listed in tables 25 and 26, respectively.

—Mean HDL-Cholesterol Level and Adherence to Fat-Controlled Diet*

Dietary Adherence	Plasma HDL-Cholesterol, mg/dl			
	Baseline, Mean	Annual Visit, Mean	Increase	
			Mean	SD
Excellent (N = 25)	48.4	52.2	3.8	13.5
Good (N = 71)	47.1	51.1	4.0†	10.0
Fair (N = 40)	45.7	45.7	0.0	12.3
Poor (N = 11)	47.6	48.0	0.4	7.5

*Degree of adherence to standard fat-controlled diet designed to lower serum cholesterol concentration was based on self-reported dietary patterns (N = 147). HDL indicates high-density lipoprotein.
†$P < .05$.

Table 25. Data from MRFIT Study (124).

Four Year Data (mean ± SEM)

Data	Group		Significance Level of Differences
	Treated	Control	
HDL cholesterol (mg/dl)	50.09 ± 2.65	42.22 ± 1.33	$p < 0.01$
"HDL cholesterol ratio"*	24.27 ± 1.85	14.36 ± 0.65	$p < 0.001$
Total cholesterol (mg/dl)	263.2 ± 6.11	341.2 ± 6.70	$p < 0.001$
LDL cholesterol (mg/dl, calculated†)	187.0 ± 5.65	258.0 ± 8.10	$p < 0.001$
Triglycerides, fasting (mg/dl)	129.3 ± 12.39	200.2 ± 15.05	$p < 0.01$
Serum uric acid (mg/dl)	4.77 ± 0.16	5.40 ± 0.14	$0.05 < p < 0.10$
Relative body weight (weight (kg)/height^2)	23.28 ± 0.52	25.65 ± 0.52	$p < 0.01$

* HDL cholesterol ratio: HDL cholesterol X 100 to Total cholesterol − HDL cholesterol.
† According to the equation: LDL cholesterol = Total cholesterol − HDL cholesterol − Triglycerides/5.

Table 26. Data from Oslo Heart Study (125).

Diets very rich in polyunsaturated fats ($^P/_S = 4$) have caused up to 33% reductions in HDL-C but such diets are not therapeutic (74).

High carbohydrate diets produce falls in plasma HDL-C concentrations (63, 75,126,127). Both sucrose and glucose produce the same effect (127). Not only is HDL changed in composition by carbohydrate feeding (127) but its catabolic rate is increased by 39% while its synthetic rate is unchanged (75).

Vegetarians consume very low cholesterol-containing diets enriched in polyunsaturated fat. Their HDL-C levels are lower than controls consuming standard diets but their total cholesterol, LDL cholesterol and VLDL-triglyceride levels are also much reduced from control levels. Overall, therefore, their total lipoprotein profiles appears to put them at low risk for developing atherosclerosis (128).

The feeding of dietary fiber (plant cell wall material) under controlled metabolic ward conditions did not affect HDL-C concentrations (129).

Cholesterol feeding raises HDL-C concentrations (129) and part of the rise may be due to the increase in HDL_c (or HDL_1) concentrations discussed earlier (95).

4. HDL and Alcohol: The fact that alcohol increases HDL levels has been well documented (130-135). The effect appears dose-dependent as demonstrated by the Cooperative Lipoprotein Phenotyping Study (132), and representative data showing this effect is given in Table 27 (132).

Table 27. Mean blood levels of lipids in relation to alcohol consumption in subjects aged 50-69. (Framingham Men).*

Alcohol (oz/wk)	No. of Subjects	Plasma Cholesterol				Plasma Triglyceride
		Total	HDL	LDL	VLDL	
		mean levels (mg/dl)				
Total	393	220	46	140	34	138
0	111	221	41	144	36	135
1-3	112	214	45	136	33	132
4-9	111	220	47	145	28	127
10-19	44	226	50	137	39	162
20+	15	232	58	123	50	213

Modified from Ref. 132.

While it is tempting to consider alcohol in the treatment of low HDL-C levels, one must remember that alcohol may increase plasma triglyceride levels (136,137) and it also contributes significantly to caloric intake. In the MRFIT study (121), persons reporting a marked decrease in alcohol intake had a slight decrease in HDL-C levels. The role of alcohol as a cardiac risk factor is still not clearly defined (138).

5. HDL and exercise. A number of studies have shown that vigorous physical exercise is associated with increased HDL-C levels (139-143). The amount of exercise needed is difficult to predict but endurance training seems to be most beneficial.

Individuals running more than 15 miles per week show significant elevations in their HDL-C levels (Table 28) (142).

The HDL-C levels of sprinters, in contrast, are no different from controls (Table 29) (143).

Mean Plasma Lipid and Lipoprotein Cholesterol Concentrations (mg/100 ml) for Runners and Control Subjects

Age Group	Triglycerides	Total Cholesterol	LDL Cholesterol	HDL Cholesterol	VLDL Cholesterol*
35–39					
Runners	71 ± 33§ (9)†	183 ± 23‡	115 ± 19‡	59 ± 10‖	9 ± 7
Controls	120 ± 78 (138)	202 ± 36	135 ± 23 (29)	43 ± 9	24
40–49					
Runners	68 ± 19‖ (22)	207 ± 19	133 ± 19	64 ± 11‖	11 ± 4
Controls	151 ± 99 (310)	212 ± 37	145 ± 37 (55)	41 ± 9	30
50–59					
Runners	74 ± 30‖ (10)	198 ± 23	116 ± 20	70 ± 19‖	10 ± 12
Controls	152 ± 126 (295)	212 ± 33	136 ± 28 (63)	44 ± 11	30
35–59					
Runners	70 ± 25‖ (41)	200 ± 23‡	125 ± 21§	64 ± 13‖	11 ± 6
Controls	146 ± 108 (743)	210 ± 35	139 ± 31 (147)	43 ± 10	29

Values are means ± SD.

*Control group values estimated as triglyceride divided by five.

†Figures in parentheses are numbers of subjects included for runners, control group A (triglycerides and total cholesterol), and control group B (lipoprotein cholesterol).

‡$0.01 < p < 0.05$ for runners versus control subjects.

§$0.001 < p < 0.01$ for runners versus control subjects.

‖$p < 0.001$ for runners versus control subjects.

Table 28.

Serum Lipid and Lipoprotein Concentrations in Runners and Controls (Mean ± SEM)

	Serum		VLDL		LDL		HDL	
Subjects	Triglyceride (mM)	Cholesterol (mg/dl)	Triglyceride (mM)	Cholesterol (mg/dl)	Triglyceride (mM)	Cholesterol (mg/dl)	Triglyceride (mM)	Cholesterol (mg/dl)
Males								
Sprinters	1.28 ± 0.7	200 ± 12	0.74 ± 0.17	15 ± 4	0.29 ± 0.03	128 ± 12	0.17 ± 0.02	50 ± 2
Long distance runners	0.89 ± 0.1	213 ± 15	0.48 ± 0.06	12 ± 2	0.25 ± 0.02†	139 ± 12	0.15 ± 0.01	66 ± 2†
Controls	1.00 ± 0.1	186 ± 12	0.51 ± 0.10	15 ± 4	0.34 ± 0.02	128 ± 12	0.15 ± 0.01	47 ± 2
Females								
Long distance runners	0.82 ± 0.1	201 ± 4	0.39 ± 0.08	8 ± 2	0.28 ± 0.01	116 ± 4	0.15 ± 0.01	74 ± 3*
Controls	0.84 ± 0.05	178 ± 8	0.42 ± 0.04	12 ± 1	0.26 ± 0.02	108 ± 8	0.16 ± 0.01	61 ± 3

*$p < 0.05$, †$p < 0.01$ for the difference from respective control group.

Table 29.

The lipoprotein lipase (LPL) activity per gram of both adipose tissue and muscle is much higher in long distance runners than in either controls or sprinters (Table 30). If the LPL activity is estimated for whole body adipose tissue and skeletal muscle, the long distance runners again appear to have much more enzyme activity than either the sprinters or the controls (Table 31) (143). This data is consistent with the notion that the high

Lipoprotein Lipase (LPL) Activity in Heparin Eluates of Skeletal Muscle and Adipose Tissue of Runners and Controls (Mean ± SEM)

Group	n	Adipose Tissue (LPL) (μmoles FFA · h^{-1} · g^{-1})	Skeletal Muscle (LPL) (μmoles FFA · h^{-1} · g^{-1})
Males			
Sprinters	8	2.37 ± 0.30	0.82 ± 0.14
Long distance runners	12	6.10 ± 1.70*	1.46 ± 0.14†
Controls	10	2.22 ± 0.29	0.85 ± 0.17
Females			
Long distance runners	6	11.4 ± 2.2	1.39 ± 0.10†
Controls	16	7.94 ± 1.02	0.90 ± 0.09

*$p < 0.05$

†$p < 0.01$ for the difference from respective control group.

Table 30.

Estimated Lipoprotein Lipase Activity of Whole Body Adipose Tissue and Skeletal Muscle (Mean ± SEM)‡

Subjects	Adipose Tissue (mmoles FFA · h^{-1})	Skeletal Muscle (mmoles FFA · h^{-1})	Total
Males			
Sprinters	38.1 ± 4.3	21.5 ± 3.6	59.6 ± 4.9
Long distance runners	87.4 ± 23.4*	35.5 ± 3.6*	125.4 ± 22.3†
Controls	34.5 ± 5.4	21.3 ± 4.6	55.8 ± 8.5
Females			
Long distance runners	170.8 ± 47.7	25.1 ± 2.1†	195.9 ± 48.9
Controls	105.0 ± 17	15.8 ± 1.6	127.3 ± 17.9

*$p < 0.05$, †$p < 0.01$ for the difference from respective control group.

‡The figures were obtained by multiplying the LPL activity per gram by the estimated weight (in grams) of whole body adipose tissue[22] and skeletal muscle[23] mass. The estimates are based on the assumption that LPL activity of the biopsy samples is representative of the whole tissues.

Table 31.

HDL levels observed with vigorous exercise are the result of accelerated chylomicron and VLDL metabolism.

6. HDL and Cigarette Smoking. Cigarette smoking is negatively associated with HDL-cholesterol levels (21,121,144-145). Cross-country skiers who smoke have lower HDL-C levels than those who do not smoke (144). In the MRFIT program, the HDL levels rose slightly in those individuals who reduced their cigarette consumption (121).

7. HDL Changes During the Menstrual Cycle. Data is sparse. In one study, a small increase in HDL_2 was noted at the time of ovulation (146), whereas in another study, HDL did not change throughout the menstrual cycle (147). If changes do occur, they are probably too small to be of clinical significance.

8. Seasonal Variation in HDL. In one study, the HDL-C levels appeared to drop significantly in March and then gradually increase through May. Peak

levels were reached in late June or early July (120). The study was not continued beyond the 5-month period. More studies are needed before the significance of this finding can be fully evaluated.

9. HDL Levels in Different Populations.

Greenlandic West Coast Eskimos have higher HDL levels than Danish controls. Eskimos living in Denmark have lipoprotein levels similar to the Danes (148). Dietary and environmental factors rather than genetic factors are thought to account for the differences.

In Jamaica, rural hill farmers had higher HDL-C levels than urban businessmen (149). The differences are thought to be environmental.

In Evans County, blacks have much higher HDL-C levels than whites (adults) (150). Similar changes are observed in children (114,116).

In New Zealand, adolescent Maoris have lower HDL-C levels than non-Maoris (151). CHD is apparently higher in Maoris than in non-Maoris.

A black African tribe in Western Transvaal is free of coronary heart disease. Their HDL cholesterol levels are high and make up from 45-55% of the total plasma cholesterol (152).

The Tarahumara Indians of Mexico have rather low HDL-C levels (25 ± 7 mg/dl in the total sample) but they also have low total cholesterol (133 mg/dl) and LDL-C (87 ± 24 mg/dl) levels (153). The total plasma cholesterol level in this group correlated positively with the dietary cholesterol intake, the first time that this association has been shown in man. As anticipated, this group of Indians is free of coronary heart disease.

The above observations are curiosities at present but they may eventually be of some help in defining the importance of HDL in atherosclerosis.

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VII. Effects of Drugs on HDL Levels.

A variety of drugs appear to change HDL-C concentrations in plasma (Table 32).

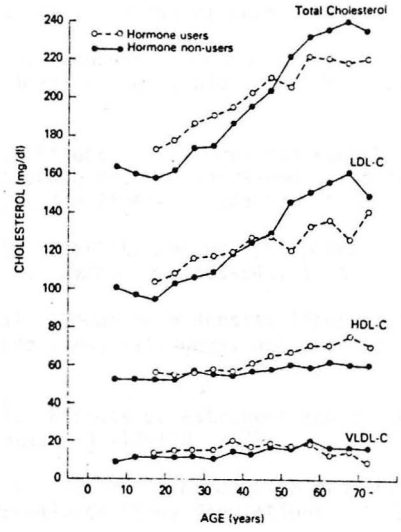
Table 32. Effect of Drugs on Plasma HDL Levels

Change from Baseline	
Increase	Decrease
Clofibrate (63,67,68)	Androgens (157,158,166)
Nicotinic Acid (75,154)	Oxandrolone (167,168)
Estrogens in men (155,156)	Progestins (162)
Estrogens in women (157-163)	Oral contraceptives (162,164,165)
Oral contraceptives (159-163)	Propanolol (171)
Vitamin C (169)	Hydrochlorthiazide plus propanolol (172)
Phenytoin (170)	
Chlorinated hydrocarbons (173)	

Clofibrate and nicotinic acid will increase HDL levels in some patients, usually in association with a hypotriglyceridemic effect. Nicotinic acid produces changes in both HDL composition and metabolism (75,154).

Exogenous estrogens increase plasma HDL levels in both sexes and it is also worth noting that plasma triglyceride levels are raised by estrogen. Thus, while HDL-cholesterol and plasma triglyceride are usually inversely related, estrogen treatment causes both lipid levels to increase in the blood. Alcohol may produce similar changes.

Oral contraceptives have been reported to increase or decrease HDL levels and both observations are valid since the net effect on HDL-C depends on the ratio of estrogen-to-progestin in the formulation (161). Progestins cause the HDL levels to drop (161). Figure 28 summarizes the results of the LRC prevalence study regarding lipid and lipoprotein levels in hormone users and non-users (163).



Mean plasma total cholesterol, LDL-C, HDL-C and VLDL-C by 10-year age groups in 732 females taking and 2601 not taking sex hormones. LRC Prevalence Study, Visit 2 random sample.

Figure 28.

Androgens and the anabolic steroid, oxandrolone (Anavar), lower HDL-C levels.

Propanolol alone or in combination with hydrochlorothiazide lowers plasma HDL levels. The effect of hydrochlorothiazide alone has not been determined.

Chlorinated hydrocarbons are usually insecticides and accidental exposure to these agents is associated with high HDL levels. It has been suggested that these agents might serve as prototypes for compounds eventually developed as therapeutic agents for the treatment of low HDL levels (174).

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VIII. HDL in disorders other than primary lipoproteins abnormalities.

HDL-cholesterol levels and, in some cases, HDL composition are altered in certain disorders (Table 33).

Table 33. Non-lipoprotein disorder in which HDL levels are altered.

Diabetes mellitus (175-182)
Renal Disease (183-196)
Cholestatic Liver Disease (197,198)
Connective Tissue Diseases (199)
Friedreich's Ataxia (200)
Familial Spastic Ataxia (200)

Only the two most common problems -- diabetes mellitus and renal disease -- will be summarized here.

A number of reports indicate that HDL levels are reduced in patients with diabetes mellitus. Authors have shown negative correlations between HDL-C concentrations and both serum glucose (175) and glycosylated hemoglobin levels (177). Other investigators have not confirmed this association (178,179). In one study, HDL levels were low in maturity-onset diabetics but normal in juvenile-onset diabetics (179). In a small series of patients, diabetics treated with oral agents had lower HDL levels than those treated with insulin (176). It is difficult to draw any conclusions from these studies since diabetes mellitus is a heterogeneous group of disorders and study populations have been even more mixed. Nikkila has studied well-classified groups of diabetics and he finds that insulin-treated diabetics (n = 91, ages 35-55) may actually have slightly higher-than-average HDL levels (181). Diabetics with ketoacidosis have reduced HDL-C levels as do non-insulin requiring diabetics with hypertriglyceridemia (180). Non-insulin-requiring diabetics without hyperlipidemia have essentially normal HDL-C levels (180). The correlation of HDL-C levels with adipose tissue lipoprotein lipase activity is moderately positive, suggesting that triglyceride metabolism probably influences the HDL-C levels, but it is also likely that insulin influences the synthesis and secretion of HDL particles (180). When the prevalence of vascular disease among 154 diabetic subjects was analyzed in relation to serum lipoprotein levels, positive correlations were observed between LDL levels and vascular disease while negative correlations were observed between HDL levels and vascular disease. The negative HDL correlation did not apply to all diabetic groups whereas the positive LDL correlation did, suggesting that there is a stronger association between vascular disease and LDL rather than HDL in diabetes (182).

HDL and the other lipoproteins have been extensively studied in relation to renal disease. Lewis and co-workers first demonstrated reduction in HDL levels following nephrectomy and its eventual restoration after renal transplantation (183). Ibels et al. found high triglyceride and low HDL levels in uremic patients either before or during chronic hemodialysis but the HDL levels became normal after transplantation (184). Most studies since that time have consistently found high triglyceride and low HDL-C levels in patients with renal disease. Since the abnormal lipoprotein patterns have been similar irrespective of the disease process in the kidney, it is assumed that uremia, per se, produces the lipid abnormalities by mechanisms still unknown (187,193,195). While one group of investigators have not found much improvement in low HDL levels during chronic hemodialysis or after renal transplantation (186) (Table 34), others have observed considerable improvement during chronic hemodialysis (Fig. 29) (188) or after successful renal transplantation (194).

Low-Density-Lipoprotein (LDL) and High-Density-Lipoprotein (HDL) Cholesterol Concentrations (Mean ±S.D.) in Normolipidemic Male and Female Control Subjects and in Undialyzed, Dialyzed and Renal-Transplant Patients Arranged According to Lipoprotein Phenotype.

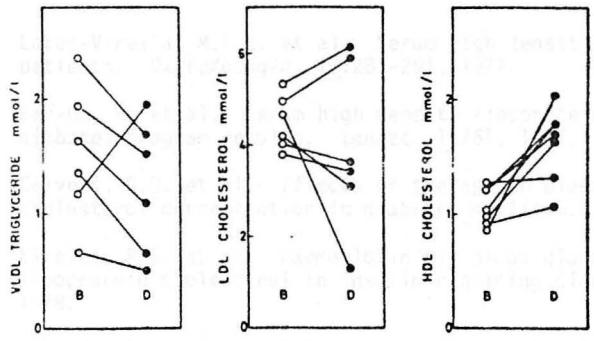
Group	No. in Group	LDL Cholesterol	HDL Cholesterol
		mg/100 ml	
Controls	430	119±28	50±11
Uremic:			
Undialyzed	13	126±47 (132±31)*	35±23‡ (49±16)
Dialyzed	14	115±30 (112±33)	38±18‡ (42±14)
Transplant:			
Entire group	23	189±56†	36±13†
Subgroups:			
Normolipidemic	5	124±22 (100±20)	39±13 (57±14)
Type IIa	2	244±8 (226±13)	41±13 (47±12)
Type IIb	8	237±47 (225±75)	34±12 (43±12)
Type IV	8	167±19 (129±37)	34±15 (45±15)

*Figures in parentheses represent values for nonuremic controls matched for sex & whole-plasma triglyceride & cholesterol levels.

†P<0.001 vs control.

‡P<0.005 vs control.

Table 34.



Serum concentrations of VLDL triglyceride, LDL cholesterol and HDL cholesterol in six patients with end-stage uremia before (*B*) and during (*D*) treatment with regular hemodialysis. Means± S.E.M. are given in Table I.

Figure 29.

Although it has been argued that the low HDL-C levels are somehow related to the high triglyceride levels in the uremic state, it is clear that low HDL-C levels can be seen in otherwise normolipidemic individuals (Table 35) (192).

Serum Triglyceride, Cholesterol and High-Density Lipoprotein (HDL) Cholesterol Levels in 21 Patients on Chronic Hemodialysis and 11 Normal Subjects (Mean ± S.D.).

Group	Triglyceride	Cholesterol	HDL Cholesterol
	mg/dl	*mg/dl*	*mg/dl*
Normal subjects	111±35	225±29	52±9
Patients			
Entire group (21)*	191±89 (P<0.01)†	263±82 (NS)‡	26±13 (P<0.001)
Type IV (7)	262±98 (P<0.001)	212±13 (NS)	26±11 (P<0.001)
Type IIa (4)	125±21 (NS)	338±60 (P<0.001)	31±16 (P<0.01)
Type IIb (4)	231±66 (P<0.01)	367±53 (P<0.001)	21±4 (P<0.001)
Normolipidemic (6)	127±19 (NS)	199±32 (NS)	28±18 (P<0.01)

*Figures in parentheses denote no. in group.
†P values refer to patients vs normal subjects. ‡Not significant.

Table 35.

The mechanisms responsible for the lipoprotein abnormalities in uremia are not well understood. It has been proposed that the low lecithin-cholesterol acyltransferase (LCAT) activity found in chronic uremics may result in incomplete HDL production with resultant low HDL-C levels (196).

Whether or not the low HDL-C levels in uremia contribute to the apparent increase in atherosclerosis found in these patients must await clarification of HDL physiology.

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IX. HDL and Atherosclerosis - clues from experiments of nature.

Several genetic disorders of lipoprotein metabolism are characterized by marked alterations in HDL levels and composition. It is instructive to review these abnormalities for clues concerning the role of HDL in atherosclerosis. Those disorders we will consider are Familial Hyperalphalipoproteinemia, Tangier Disease, and Familial Lecithin:Cholesterol Acyltransferase (LCAT) Deficiency. Abetalipoproteinemia (ABL) is also associated with alterations in HDL composition but since all the other plasma lipoproteins are absent in this disease, a study of ABL will not provide many clues about the interaction of different lipoproteins in the pathogenesis of atherosclerosis (201,202).

1. Familial Hyperalphalipoproteinemia. This disorder more closely resembles a laboratory curiosity than it does a clinical disorder. The syndrome was described by Glueck and co-workers, who evaluated several families for this apparent abnormality using an HDL cholesterol of 70 mg/dl as the upper normal limit (90th%) (203). Affected individuals have a significantly higher mean HDL-cholesterol level (82 mg/dl) than do the control subjects (56 mg/dl) and the hyperalphalipoproteinemia appears to be transmitted as an autosomal dominant trait (203,204). Concentrations of the other lipoproteins are normal (although the LDL-C levels are on the low side of normal). When longevity, morbidity, and death from myocardial infarction were examined in 18 kinships with this syndrome, life expectancy for males and females was 5 and 7 years longer, respectively, than that expected by population statistics from U.S. white populations (205). For that reason, it has been called a longevity syndrome (205). The composition of HDL in the affected individuals is apparently normal (206) and the physiologic basis for the hyperalphalipoproteinemia is unknown.

   Problems in interpretation of these data have recently been summarized and the effects of environmental factors, diet, and sex have not been adequately evaluated (207). Thus, the acceptance of hyperalphalipoproteinemia as a

distinct autosomal dominant genetic entity is still tentative. In any case, proponents of the HDL hypothesis use this syndrome to support their position.

In the same vein, several recent reports have examined the inheritance patterns of HDL levels in myocardial infarction survivors and their first-degree relatives (208-210).

In two of the studies, a small but statistically significant parent-offspring correlation of the HDL-C level was observed (208.209). In the third study, male relatives of myocardial infarction survivors were found to have significantly lower HDL-C levels than controls. However, major dominant gene effects were not observed in any of these studies.

2. Tangier Disease (Familial HDL Deficiency) (30,211-214). Tangier Disease is a rare disorder of lipoprotein metabolism thought due to a mutant autosomal gene. The homozygote for this disease manifests the following abnormalities: 1) low plasma cholesterol and normal-to-elevated plasma triglyceride concentrations; 2) absence of HDL and especially the major apoprotein, apoA-I; 3) widespread tissue storage of cholesteryl esters; and 4) neuropathy. Of most interest to us is the relationship of the nearly absent HDL levels to the tissue storage of cholesteryl ester for if the HDL hypothesis is correct, one might reasonably expect that Tangier homozygotes would suffer from rapidly progressive atherosclerosis. The lipid deposition in this disease, however, is confined largely to cells in the recticuloendothelial system (RES) (lymph nodes, thymus, bone marrow, rectal mucosa, and Schwann cells). Most cells of the body (e.g., parenchymal cells, blood cells, endothelial cells, smooth muscle cells of arteries, etc.) do not accumulate lipid in this disease. The reason for the lipid deposition in the RES has not yet been clarified.

   In any case, the arteries of Tangier homozygotes are not clogged with atheromas and the clinical histories of most patients do not reflect problems with premature atherosclerosis (30).

   These findings should be contrasted with those of homozygous Familial Hypercholesterolemia (215). Epidemiologists have long recognized that total plasma cholesterol levels are positively associated with increasing cardiovascular risk and the total plasma cholesterol accurately reflects and LDL-cholesterol in most cases. These epidemiological findings are given added credence based on the findings in Familial Hypercholesterolemia (FH). Patients heterozygous for this condition have total and LDL cholesterol levels about 2.5-fold greater than normal and have myocardial infarctions in their 30's and 40's. Homozygotes for FH have plasma total and LDL cholesterol levels 6-8 fold above normal and typically experience myocardial infarctions in the first decade of life and rarely live beyond the age of 30 years.

   Thus, these two experiments of nature support our concepts that high LDL levels are harmful but they challenge our notion that low HDL levels are harmful. Proponents of the HDL hypothesis argue that in Tangier Disease, the HDL is available just long enough to do its job (remove tissue cholesterol except from RES) and then it quickly vanishes from the plasma because it is structually unstable. Obviously this suggestion is difficult to either prove of disprove.

3. Familial Lecithin:Cholesterol Acyltransferase Deficiency (216). Familial LCAT deficiency is a rare autosomal recessive disorder characterized by corneal opacities, anemia, proteinuria, very low plasma cholesteryl ester levels and absent or nearly absent levels of plasma LCAT activity. One might simplistically expect tissue cholesterol deposition to be excessive in this disease if the LCAT deficiency does not allow HDL to carry cholesterol efficiently as cholesteryl ester. These patients do appear to have early atherosclerosis involving especially the aorta, large arteries, renal arteries, and renal arterioles.

   Unfortunately the issue is more complex in this disorder. Many of the patients have proteinuria and eventually develop hypertension and renal failure. Many of them are also severely hypercholesterolemic. The LCAT deficiency is also associated with structural abnormalities in the other lipoproteins which may render them more atherogenic. Thus, Familial LCAT deficiency is not of much help in providing us insight into the relationship of HDL to atherosclerosis.

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X. Usefulness of the HDL-cholesterol measurement in clinical practice - an appraisal (and opinion).

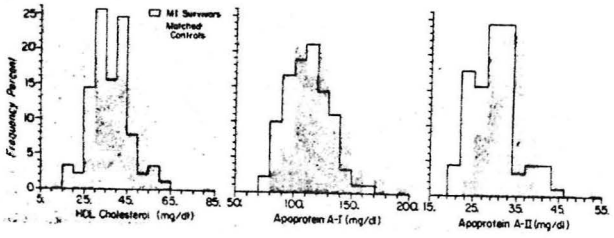
The following factors should be considered in estimating the clinical usefulness of the HDL-cholesterol measurement.

1. The negative relationship between HDL-C and CHD has been established largely in older individuals. The relationship is unknown in the 20-40 year age group.

2. The negative relationship between HDL-C and CHD does not establish cause-and-effect. Our current understanding of HDL metabolism and our observations in Tangier Disease do not provide insight into why HDL should protect against atherosclerosis.

3. When one compares the mean HDL-C in CHD victims and controls, the differences are statistically significant but small in magnitude. In the Honolulu Heart Study, for example, the CHD victims aged 50-59 had a mean HDL-C of 41.8 mg/dl while the age-matched controls had a mean value of 44.6 mg/dl. Thus the distribution of HDL-C in the two populations shows considerable overlap. This overlap is graphically illustrated in a more limited study from Seattle in which HDL-C levels (and apoprotein levels) were compared in MI survivors and controls (Fig. 28, Table 33) (119). The shaded area in Figure 28 represents the controls.

Figure 28.



Distribution of HDL cholesterol and apoproteins A-I and A-II in MI survivors and lipid-matched controls.

A-I and A-II Apoprotein and HDL Cholesterol Levels

Subject Group	Mean ± SEM	Percentile Values				
		5th	10th	50th	90th	95th
Apolipoprotein A-I levels						
Population controls	120.5 ± 1.5	90	96	121	148	158
Lipid-matched controls	121.0 ± 2.0	94	98	121	147	156
MI survivors	111.6 ± 2.0	83	87	110	138	140
Apolipoprotein A-II levels						
Population controls	33.4 ± 0.4	26	28	33	40	43
Lipid-matched controls	33.2 ± 0.6	25	27	33	38	44
MI survivors	29.1 ± 0.5	22	23	29	37	40
HDL Cholesterol levels						
Population controls	45.0 ± 0.9	29	32	44	59	67
Lipid-matched controls	43.4 ± 1.2	27	32	43	56	63
MI survivors	38.9 ± 1.0	22	27	39	52	57

Table 33.

4. It has been pointed out that under the best laboratory conditions, the HDL cholesterol varies by ± 5 mg/dl which is uncomfortably large when significant changes in risk occur with 10 mg/dl changes. Variation in routine laboratories is likely to be even greater.

5. Even when one knows the HDL-C level to be low, there are no specific therapeutic actions one would take beyond those already recommended for risk factor modification (e.g., treatment of hypertension, treatment of hyperlipidemia, low-fat diet therapy, weight loss, discontinuence of cigarettes). Alcohol therapy and jogging are likely to be of limited use at best. Estrogens raise the HDL-C level in men but also increase morbidity and mortality from cardiovascular disease (219).

Thus, in the individual patient, the measurement of HDL-C does not appear to be very useful. The diagnostic utility of the test was recently analyzed by investigators from Johns Hopkins University, and they reported their results in a letter to the *New England Journal of Medicine* (217). These workers concluded that the data from the American Studies allowed the physician a confidence in diagnosis about equal to that in a toss of a coin. The body of the letter is reproduced below.

To the Editor: We advise forbearance in the use of high-density lipoprotein cholesterol levels for the prediction of ischemic heart disease, since the diagnostic utility of the test remains unexamined. Our critical review of the few articles on this subject reveals three prospective and six retrospective studies in which the data allow calculation of the diagnostic power of this index as a univariate parameter.

We analyzed the data in these studies in two ways. First of all, we calculated the sensitivity, specificity and summed percentage of false results (percentage wrong) at three disease-prevalence levels,

Table 1. Summary of Findings for a Prevalence Level of 0.5.

Source	Subjects	Cutoff	Sensitivity	Specificity	% Wrong	Likelihood Ratio	Posterior Probability
		mg/dl					
Nikkila[2]	58	45	0.71	0.82	24	3.9	0.4 0.80 0.29
Miller[3]	6,595	30	0.76	0.84	20	4.8	0.3 0.83 0.23
Albers[4]	180	40	0.62	0.54	42	1.3	0.7 0.57 0.42
Gordon[5]	1,025	45	0.70	0.50	40	1.4	0.6 0.58 0.37
Toss of coin	Large	Heads	0.50	0.50	50	—	

with the "cutoff" value for test positivity set at each prevalence level to minimize the percentage wrong. Secondly, we used a Bayesian method of analysis to derive likelihood ratios and posterior probabilities for test outcomes on either side of the "cutoff" value. Table 1 summarizes our findings for a prevalence level (i.e., prior probability) of 0.5. All the data presented are from studies that indicate the greatest diagnostic utility (Nikkila and Miller) or that represent North American findings (Albers and Gordon).

It is readily apparent that the data from American studies allow the physician a confidence in diagnosis about equal to that in a toss of a coin. Also noteworthy are the modest displacements of the posterior from the prior probability. On the other hand, the Scandinavian studies suggest that, for that population, some diagnostic value may exist. For all the studies reviewed, at a disease prevalence of 0.1, the sensitivity is <0.2, specificity 1.0, and percentage wrong 8 to 10; at a disease prevalence of 0.9, the sensitivity is 1.0, specificity <0.2, and percentage wrong 8 to 10. It is obvious that for use as a screening test, in which the prevalence is <0.1, sensitivity is very low. Though these studies are not homogeneous in study design, disease criteria, biochemical methodology or consideration of confounding variables, all indicate that there is no foundation for approval of the uncritical use of the high-density lipoprotein cholesterol level as a routine diagnostic test.

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Havel has argued that there is one important clinical use for the HDL-C measurement. In about 6% of women and 1% of men, elevated plasma cholesterol levels are caused by unusually high HDL-C levels (greater than 100 mg/dl) (218). Thus a person with a total plasma cholesterol of 270 mg/dl but an HDL cholesterol of 100 mg/dl would have 170 mg of cholesterol in LDL + VLDL whereas most such people would have an HDL cholesterol closer to 50 mg/dl, leaving 220 mg in LDL + VLDL. Because these differences would affect therapeutic recommendations, Havel suggests that HDL cholesterol should be measured at least once in individuals whose plasma cholesterol level is in the range where long-term treatment is being considered.

Since the number of such individuals in small and the reliability of the HDL measurement in routine clinical labs is limited, we advise against the routine measurement of the HDL cholesterol at this time.

Cardiac risk factors should ideally be identified in people less than 50 years of age where attempts to modify them would be expected to retard the development of atherosclerosis. In this age group, the total cholesterol and triglyceride measurements in fasting subjects remain useful, reliable, and relatively inexpensive in the evaluation of hyperlipidemia as a risk factor.

The data from the Framingham Study relating plasma lipid and lipoprotein levels levels to cardiovascular risk are provided below in tabular form for clinical reference.

TABLE 1

Total Cholesterol	
300 mg/dl	Almost three times standard risk: very probably a type of hyperlipoprotein emia.
260 mg/dl	Twice the standard risk: possibly a type of hyperlipoproteinemia
234 mg/dl	1.4 x standard risk: average cholesterol of CHD cases in the Framingham study
225 mg/dl	Standard risk: average cholesterol
220 mg/dl	0.95 x standard risk: average cholesterol of non-CHD cases in the Framingham study
200 mg/dl	0.9 x standard risk
185 mg/dl	0.8 x standard risk
150 mg/dl	Probable cholesterol threshold for CHD

TABLE 2

HDL Cholesterol

The following factors indicate the increase or decrease in risk of CHD by level of HDL and sex.

HDL Cholesterol	Male	Female
25	2.00	---
30	1.82	---
35	1.49	---
40	1.22	1.94
45	1.00	1.55
50	0.82	1.25
55	0.67	1.00
60	0.55	0.80
65	0.45	0.64
70	---	0.52
75	Longevity Syndrome	

TABLE 3

If both HDL and total cholesterol values are known, the risk of CHD can be more accurately assessed by the ratio of these two values as follows (Castelli, 1977):

	RISK	TOTAL CHOLESTEROL/ HDL CHOLESTEROL
MEN	½ Average	3.43
	Average	4.97
	2X Average	9.55
	3X Average	23.99
WOMEN	½ Average	3.27
	Average	4.44
	2X Average	7.05
	3X Average	11.04

TABLE 4

If the value for triglycerides is also known, LDL cholesterol can be calculated by Friedewald's equation and the most difinitive assesment of CHD risk available from a lipid profile can be determined:

	RISK	LDL CHOLESTEROL/ HDL CHOLESTEROL
MEN	½ Average	1.00
	Average	3.55
	2X Average	6.25
	3X Average	7.99
WOMEN	½ Average	1.47
	Average	3.22
	2X Average	5.03
	3X Average	6.14

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