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Assessment of Lipids and Lipoproteins

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Introduction

The circulating lipids include free cholesterol, cholesterol esterified with long-chain fatty acids, triacylglycerols (triglycerides, TG), phospholipids, and unesterified or free fatty acids. Lipids are transported in the blood plasma in the form of lipoproteins. The lipoproteins include:

- Chylomicrons
- Very low-density lipoproteins (VLDL)
- Intermediate-density lipoproteins (IDL, beta-VLDL)
- Low-density lipoproteins (LDL)
- High-density lipoproteins (HDL)

The chemical and physical properties of the lipoproteins[1] are shown in Table 26.1. This section summarizes biological factors influencing lipid and lipoprotein levels, describes methodology for assays in common clinical use in laboratories or health care facilities, and provides data on the range of normal values.

Cholesterol

Cholesterol is synthesized by all animal cells (endogenous) and by no plants. It also is derived from animal products in the diet (exogenous). Food sources are listed in Table 51.6 in Section 51. Circulating cholesterol levels vary with age, increasing in men from puberty to the fifth decade of life, and in women until the seventh decade[2] (Table 26.2). Levels in women are lower than in men from age 30 to 50. Mean cholesterol levels vary

TABLE 26.1

Plasma Lipoproteins in Humans[a]

Class	Particle Diameter (nm)	Flotation Density	Electrophoretic Mobility	Major Apoproteins	Chemical Composition, %				
					Surface			Core	
					Proteins	Phospholipids	Cholesterol	Cholesterol Esters	Tryglycerides
Chylomicrons	80–500	<0.95	α2	B, E, A-1 A-IV, C	2	7	2	3	86
VLDL	30–80	0.95–1.006	pre-β	B, E, C	8	18	7	12	55
IDL	25–35	1.006–1.019	slow pre-β	B, E	19	19	9	29	23
LDL	18–28	1.019–1.063	β	B	22	22	8	42	6
HDL_2	9–12	1.063–1.125	α1	A-I, A-II	40	33	5	17	5
HDL_3	5–9	1.125–1.210	α1	A-I, A-II	55	25	4	13	3

Note: VLDL, Very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

[a] Modified from Feldman, E.B. *Essentials of Clinical Nutrition,* F.A. Davis, Philadelphia, 1988, p. 433.

TABLE 26.2

Average Levels of Circulating Lipids[a,b]

	Total C		LDL C		HDL C		TG	
Age, yr	**mmol/L**	**mg/dl**	**mmol/L**	**mg/dl**	**mmol/L**	**mg/dl**	**mmol/L**	**mg/dl**
White Men								
15–19	3.95	152	2.42	93	1.20	46	0.77	68
20–24	4.13	159	2.63	101	1.17	45	0.88	78
25–29	4.58	176	3.02	116	1.14	44	0.99	88
30–34	4.94	190	3.22	124	1.17	45	1.15	102
35–39	5.04	194	3.41	131	1.12	43	1.23	109
40–44	5.30	204	3.51	135	1.12	43	1.39	123
45–49	5.46	210	3.67	141	1.17	45	1.34	119
50–54	5.49	211	3.72	143	1.14	44	1.45	128
55–59	5.56	214	3.77	145	1.20	46	1.32	117
60–64	5.59	215	3.72	143	1.27	49	1.25	111
65–69	5.54	213	3.80	146	1.27	49	1.22	108
70+	5.56	214	3.69	142	1.25	48	1.30	115
White Women								
15–19	4.08	157	2.42	93	1.33	51	0.72	64
20–24	4.29	165	2.65	102	1.33	51	0.90	80
25–29	4.6	178	2.81	108	1.43	55	0.86	76
30–34	4.63	178	2.83	109	1.43	55	0.82	73
35–39	4.84	186	3.02	116	1.38	53	0.94	83
40–44	5.02	193	3.17	122	1.46	56	0.77	68
45–49	5.30	204	3.30	127	1.51	58	1.06	94
50–54	5.56	214	3.48	134	1.61	62	1.16	103
55–59	5.95	229	3.77	145	1.56	60	1.25	111
60–64	5.88	226	3.87	149	1.59	61	1.18	105
65–69	6.06	233	3.93	151	1.61	62	1.33	118
70+	5.88	226	3.82	147	1.56	60	1.24	110

[a] See Table 26.1 for abbreviations of lipoproteins; C, cholesterol; TG, triglycerides.
[b] Adapted from Lipid Research Clinics Program, *JAMA* 251, 351, 1984.

between 4.2 and 6 mmol/L, depending on age and gender. Population levels have been declining over recent decades. About two-thirds of the plasma cholesterol is transported as LDL and levels of LDL cholesterol parallel those of total cholesterol (Table 26.2). HDL transports about one-quarter of the plasma cholesterol, with levels averaging about 1.17 mmol in men, and are 0.23 to 0.44 mmol higher in women (Table 26.2). Table 26.3 provides data on the upper limits of normal for LDL- and HDL-cholaterol. Table 26.4 provides data on mean values for lipids and lipoproteins in men and women from NHANES III data.[3]

Cholesterol assays and the reference method[4-7] are provided in Table 26.5. Clinical laboratories are automated for the lipid analyses. Specific methods are provided as kits by the manufacturer of the analytical instrument in use. Desktop methodologies also are available for outpatient facilities (physicians' offices, clinics) but are not as accurate or precise as the commercial or hospital laboratory procedures. The latter are regulated and supervised by accrediting organizations such as the College of American Pathologists, and laboratories are regulated by the government (CLIA).

TABLE 26.3

Levels of Circulating Lipids Warranting Attention[a,b]

	LDLC		HDLC		TG	
	75th percentile		25th percentile		90th percentile	
Age, yr	mmol/L	mg/dl	mmol/L	mg/dl	mmol/L	mg/dl
White Men						
15–19	2.83	109	1.01	39	1.41	125
20–24	3.07	118	0.99	38	1.64	146
25–29	3.59	138	0.96	37	1.92	171
30–34	3.74	144	0.99	38	2.41	214
35–39	4.00	154	0.94	36	2.81	250
40–44	4.08	157	0.94	36	2.84	252
45–49	4.24	163	0.99	38	2.84	252
50–54	4.21	162	0.94	36	2.74	244
55–59	4.37	168	0.99	38	2.36	210
60–64	4.29	165	1.07	41	2.17	193
70+	4.26	164	1.04	40	2.27	202
White Women						
15–19	2.89	111	1.12	43	1.26	112
20–24	2.07	118	1.14	44	1.52	135
25–29	3.28	126	1.22	47	1.54	137
30–34	3.33	128	1.20	46	1.58	140
35–39	3.61	139	1.14	44	1.91	170
40–44	3.80	146	1.25	58	1.81	161
45–49	3.90	150	1.22	47	2.02	180
50–54	4.16	160	1.30	50	2.14	190
55–59	4.37	168	1.30	50	2.6	229
60–64	4.37	168	1.33	51	2.36	210
65–69	4.78	184	1.27	49	2.49	221
70+	4.42	170	1.25	48	2.13	189

[a] See Table 26.1 for abbreviations.
[b] Adapted from Lipid Research Clinics Program, *JAMA* 251, 351, 1984.

Triacylglycerols (TG)

Circulating TG levels average about 1.13 mmol/L in young adults after overnight fasting. Levels increase from 50 to 75% with age, and are lower in women compared to men. (Tables 26.2 and 26.3). Median TG values range from 0.90 to 1.47 mmol/L. TG levels are labile, varying by up to 50% daily depending on the recent diet. In the fasting state, TG are transported in the VLDL, whereas chylomicrons transport newly absorbed fat. Upper limits of normal for TG are given in Table 26.3.

Triacylglycerol assays and reference method[9-11] are listed in Table 26.5.

Lipoproteins

Lipoprotein assays[12-15] are given in Table 26.5.

TABLE 26.4

Lipid Levels U.S. NHANES III Population[a,b]

Lipid Level (mg/dL)	Mean ± SD (mg/dL)
Mean total cholesterol	225 ± 45
Men	218 ± 42
Women	237 ± 47
Mean LDL-C	142 ± 37
Men	139 ± 35
Women	147 ± 40
Mean HDL-C	50 ± 16
Men	47 ± 14
Women	56 ± 17
Median TG	140 ± 120
Men	137 ± 129
Women	144 ± 108
Mean total-C/HDL-C	4.9 ± 2.1
Men	5.1 ± 1.7
Women	4.7 ± 2.6
Mean LDL-C/HDL-C	3.1 ± 1.5
Men	3.2 ± 1.2
Women	2.9 ± 1.9
Apolipoprotein A1	147 ± 27
Men	139 ± 23
Women	158 ± 29
Apolipoprotein B	116 ± 26
Men	115 ± 24
Women	119 ± 27

[a] See Table 26.1 for abbrevations.

[b] DHHS NCHS. *Third National Health and Nutrition Examinations Survey,* 1988-94, NHANES III, Hyattsville, MD, 1996.

Chylomicrons

These particles originate in the small intestine when fat is absorbed, and are absent in plasma from fasting subjects. They are visibly present in blood when TG levels exceed 7.90 mmol/L, and the refrigerated plasma may appear turbid (Figure 52.8, Section 52). At higher TG levels the standing plasma will show a creamy top layer. Chylomicrons are transported into the lymphatic system, delivered into the blood, and removed by the action of the enzyme lipoprotein lipase (LPL) to produce remnant particles that are taken up by specifc receptors in the liver. (Figure 26.1). The composition of chylomicrons is listed in Table 26.1.

VLDL

These particles are produced in the liver and result from *in vivo* synthesis from carbohydrate precursors or from free fatty acids mobilized from adipose tissue and delivered to the liver. VLDL composition is given in Table 26.1. The standing plasma begins to appear diffusely turbid when TG levels exceed 2.25 mmol/L (Figure 52.8, Section 52). Lipoprotein lipase action produces VLDL remnants, or IDL, that are rapidly removed from the blood by receptors in the liver (Figure 26.1). An assay for VLDL remnants that has been developed for research studies[12] is under consideration by laboratory manufacturers for clinical applications.

TABLE 26.5

Tests for Plasma Lipids, Lipoproteins, and Lipolytic Enzymes

Assay	Principle of Method	Reference Method	Clinical/Usual Method	Performance Criteria
Total cholesterol	Chemical	Abell-Kendall[5]		CV ≤3%
	Spectrophotometric			Bias <3%
	Modified Liebermann-Burchard[4a,b]			
	Automated enzymatic		Allain[6]	
	Colorimetric		Rautela[7]	
Triacylglycerol (TG)	Glycerol assay	Van Handel-Zilversmit[8]		CV <5%
	Spectrophotometric			Bias <5%
	Automated enzymatic			
	Spectrophotometric		Sampson[9]	
			Hagen[10]	
			Rautela[11]	
VLDL				
VLDL remnant	Ultracentrifugation		Nakajima[12]	
LDL-calculate	TC-{HDL-C + TG/5}		Friedewald[13]	CV <4%
				Bias <4%
LDL ultracentrifuge	C in d<1.006 – HDL-C	Beta-quant	Havel-Eder-Bragdon[14]	
LDL direct	Precipitation of chylomicrons, VLDL IDL, HDL by antibodies to apo-E and apo A-I		McNamara[15]	
Lp(a)	ELISA		Marcovina[17]	
HDL	Heparin-Mn or dextran-Mg precipitation of VLDL, LDL; analyze C in supernatant		Burstein[19]	CV <4%
			Warnick[20]	Bias <5%
Apo A-I	Immunoassay	Not available	Albers[24]	CV 6%
Apo B	Immunoassay	Not available	Warnick[25]	
Phospholipids	Lipid phosphorus (lipid extract)		Bartlett[26]	
Free fatty acids	Titration of extracted plasma		Dole-Meinertz[27]	
Fatty acid composition	Gas-liquid chromatography of fatty acid methyl esters		Nelson[28]	
Lipoprotein lipase (LPL)	Hydrolysis of radioactive lipid emulsion by post-heparin plasma		Olivecrona[30]	3-5% accuracy Plasma pool
Hepatic lipase (HL)	Antiserum to HL NaCl inhibition of LPL		Huttunen[31]	Values lower in women
Lecithin-cholesterol acyl transferase (LCAT)	Double antibody radioimmunoassay		Albers[32]	

Note: CV = coefficient of variation.

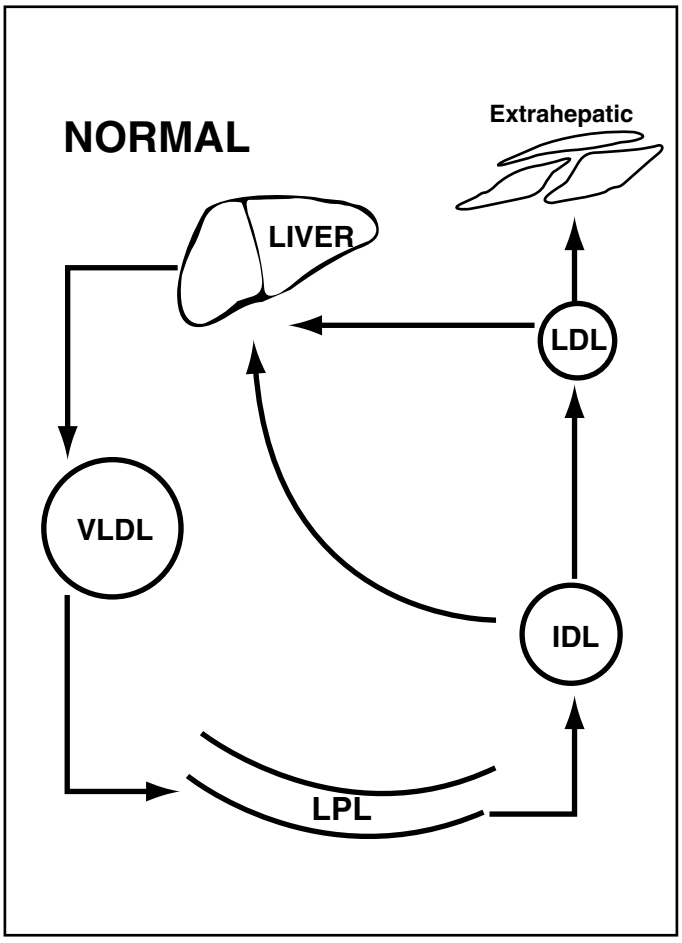

FIGURE 26.1
Production of lipoproteins, delivery into blood, and removal by tissues. See Table 26.1 for abbreviations. LPL = lipoprotein lipase.

IDL

This TG-rich particle is relatively enriched in the proportion of cholesterol esters compared to its precursor VLDL (Table 26.1). The atherogenic beta-VLDL is a related cholesterol-rich particle with interactions with the LDL and remnant receptors (Figure 26.1). This particle is evanescent in the plasma of healthy normolipidemic subjects.

LDL

This particle is the main transporter of cholesterol in the blood and is considered the most atherogenic lipoprotein. The LDL cholesterol level reflects changes in lipoprotein composition. LDL delivers cholesterol to cells and is taken up by specific cell surface receptors (Figure 26.2). LDL assays are listed in Table 26.5. LDL cholesterol may be calculated as:

$$\text{LDL} = [\text{Total cholesterol}] - [\text{HDL-cholesterol}] - [\text{TG}/5]^{13}$$

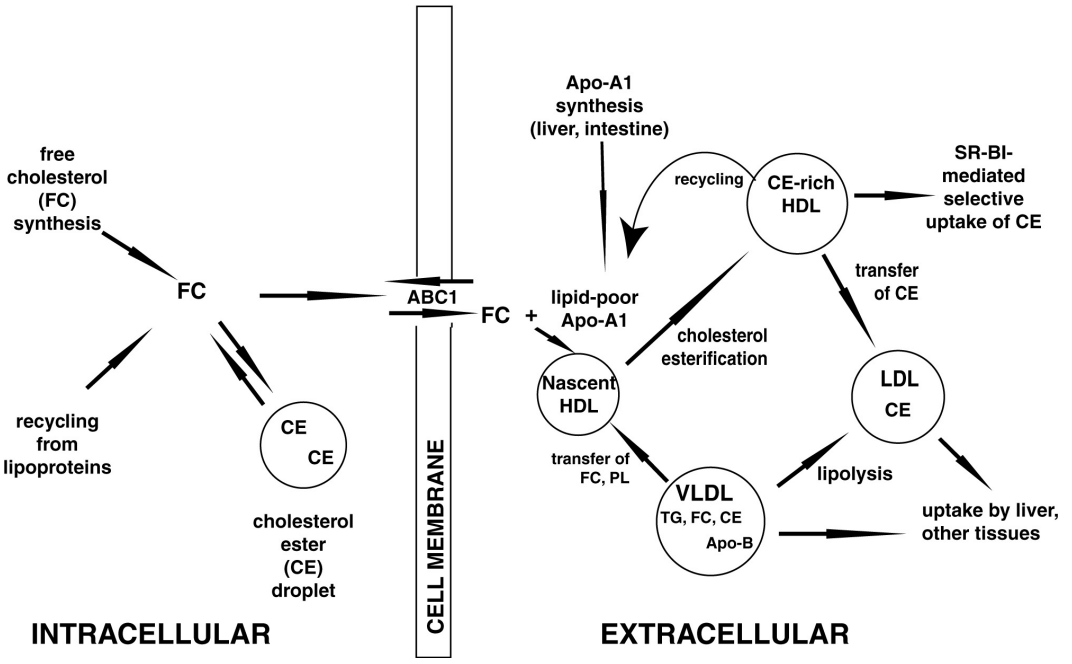

FIGURE 26.2
The generation of HDL and the interrelations of the lipoproteins, their production, and removal. ABC 1 = ATP-binding-cassette transporter. SRB 1 = Scavenger receptor binder. Adapted from Young S. G., and Fielding C. J., *Nature Genetics*, 22, 316, 1999.

Alternatively, LDL can be measured by ultracentrifugation (beta-quantification, or beta-quant)[14] or directly, using an immunologic procedure[15] (Table 26.5).

LDL particle size is variable, ranging from large and buoyant to small and dense (Table 26.6). The small dense particle, oxidative changes in LDL, and the presence of a variant of LDL, Lp(a), raise the atherogenicity of LDL. Lp(a) levels range from undetectable to 1000 mg/L. The risk of atherosclerosis increases as values exceed 300 mg/L. The Lp (a) assay[17] is listed in Table 26.5.

HDL

The HDL particle is generated by the transfer of surface lipids from TG-rich lipoproteins during lipolysis[18] (Figure 26.2). The HDL2 and HDL3 particles differ in size and composition, and particles vary in their content of the specific apolipoproteins, apo A-I and A-II (Tables 26.1 and 26.6). HDL is protective against atherosclerosis, enhancing removal mechanisms by reverse cholesterol transport. Methods of assay[19, 20] are described in Table 26.5.

Standardization of Assays

Blood samples for assays of serum or plasma lipids and lipoproteins should be obtained under standardized conditions of a stable diet, avoiding alcohol, and in the morning after an overnight fast. Serum and plasma values differ as do values of cholesterol when the

TABLE 26.6

Lipoprotein Subclasses[a]

Particle	Diameter	nm	Association with Risk of CVD
VLDL 1	Large	80	Higher
2	Medium	70	Intermediate
3	Small	60	Lower
IDL 1	Large	40	Higher
2	Small		
LDL I	Large	30	Lower
II a			
II b			
III a	Medium		Intermediate
III b			
IV a	Small		Higher
IV b		20	
HDL 2 b	Large	10	Negative risk
2 a		9	
3 a	Small	8	Positive risk
3 b		7	
3 c		6	

[a] See Table 26.1 for abbreviations.

subject is supine, sitting, or standing. A recent meal has minor effects on cholesterol and predominantly cholesterol-containing lipoproteins (LDL, HDL), but has a major influence on levels of TG and TG-containing lipoproteins (VLDL). Optimally, determinations should be carried out on plasma samples obtained by using EDTA as anticoagulant and prepared and stored carefully. The biological variation of cholesterol, within the normal range, approximates 16%.[21] Laboratory accuracy and precision should be standardized with reference materials or reference laboratories.[22] Because of variability, more than one sample of plasma or serum lipids should be drawn, with an interval of several weeks of unchanged lifestyle, and analyzed in order to evaluate lipid status or therapy.[23]

A simple clue to lipid/lipoprotein values is provided by the standing plasma test (Figure 52.8, Section 52). Plasma is refrigerated overnight and examined for turbidity. Hypercholesterolemia does not cause the plasma to become cloudy, whereas elevated TG, either as VLDL, remnants, or chylomicrons will produce diffuse turbidity with or without a creamy supernatant layer (see Section 52).

Apoproteins

The apolipoproteins or apoproteins determine the metabolic fate of the lipoprotein particles and the solubility of lipoprotein lipids in plasma. They include:

- Apo A-I
- Apo A-II
- Apo A-IV
- Apo B-100
- Apo B-48

- Apo C-I
- Apo C-II
- Apo C-III
- Apo-D
- Apo E-2
- Apo E-3 {E-phenotype: E2/2, 2/3, 2/4, 3/3, 3/4, 4/4}
- Apo E-4
- Apo-F
- Apo-G
- Apo-H
- Apo-J
- Apo (a)

Their distribution among the lipoproteins is shown in Table 26.1.

The assay methods available in some clinical laboratories[24,25] are provided in Table 26.5. The mean values in plasma are provided in Table 26.7. The apoprotein level indicates the number of lipoprotein particles in plasma (i.e., concentration). The apoprotein composition and levels are determined in some genetic and lipid laboratories using electrophoretic and immunologic methods.

Other Lipid Assays

- Phospholipids are determined by measuring lipid phosphorus after lipid extraction.[26]
- Free fatty acids in plasma can be analyzed, usually in relation to metabolic abnormalities, such as diabetes mellitus, and related to values of glucose and insulin. The assay is listed in Table 26.5.[27]
- The fatty acid composition of plasma lipids, and separated and isolated free fatty acids, cholesterol esters, phospholipids, or TG can be quantified.[28] This may be useful in the diagnosis of essential fatty acid deficiency and some inborn errors of metabolism.
- Fecal fat can be measured as free fatty acids or TG fatty acids in order to test for malabsorption syndromes.[29]

Regulators of Lipid Metabolism

Enzymes, receptors, and transporters involved in the regulation of lipid and lipoprotein metabolism are listed in Section 52, Table 52.1. Their values are determined primarily in lipid research laboratories rather than as part of the usual clinical lipid profile for patient

TABLE 26.7

Average Levels of Apoproteins in Plasma (mg/L)[a,b]

Apoprotein	Mean ± SD
A-I	1,200 ± 200 (men)
	1,350 ± 250 (women)
A-II	330 ± 50 (men)
	360 ± 60 (women)
B	1,000 ± 200
C-I	70 ± 20
C-II	40 ± 20
C-III	130 ± 50
D	60 ± 10
E	50 ± 20

[a] SD = standard deviations.

[b] From Albers, in *Eleventh International Congress of Clinical Chemistry.* Keuser, E., Giabal, F., Muller, M. M., et al., Eds., Walter de Greyter, Berlin, 1982, with permission.

assessment. Methods for the determination of post-heparin lipolytic activity, lipoprotein lipase,[30] hepatic lipase,[31] and lecithin:cholesterol acyltransferase[32] are listed in Table 26.5.

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