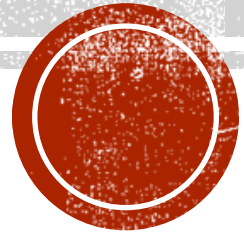
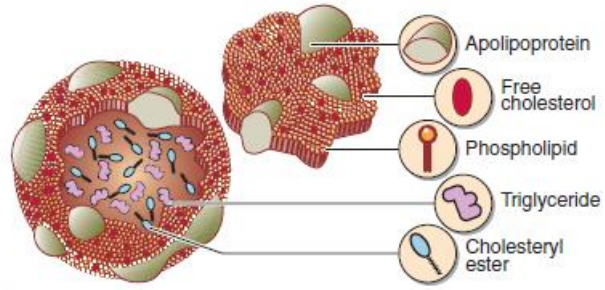
LIPID PROFILE



LIPOPROTEIN METABOLISM

- The lipoprotein system evolved to solve the problem of transporting fats around the body in the aqueous environment of the plasma.
- A lipoprotein is a complex spherical structure that has a hydrophobic core wrapped in a hydrophilic coating.
- The core contains triglyceride and cholesteryl esters, while the surface contains phospholipid, free cholesterol and proteins – the apolipoproteins.
- Cholesterol is an essential component of all cell membranes and is a precursor for steroid hormone and bile acid biosynthesis.
- Triglyceride is central to the storage and transport of energy within the body



Structure of a lipoprotein.

TYPES OF LIPOPROTEINS

Table 66.2 **The four main lipoproteins and their functions**

Lipoprotein	Main apolipoproteins	Function
Chylomicrons	B_{48}, A-I, C-II, E	Largest lipoprotein. Synthesized by gut after a meal. Not present in normal fasting plasma. Main carrier of dietary triglyceride
Very low density lipoprotein (VDL)	B_{100}, C-II, E	Synthesized in the liver. Main carrier of endogenously produced triglyceride
Low density lipoprotein (LDL)	B_{100}	Generated from VLDL in the circulation. Main carrier of cholesterol
High density lipoprotein (HDL)	A-I, A-II	Smallest lipoprotein. Protective function. Takes cholesterol from extrahepatic tissues to the liver for excretion

LIPID METABOLISM

- Lipoprotein metabolism can be thought of as two cycles, one exogenous and one endogenous, both centred on the liver. These cycles are interconnected.
- Two key enzyme systems are involved in lipoprotein metabolism, i.e.:
- Lipoprotein lipase (LPL) releases free fatty acids and glycerol from chylomicrons and VLDL into the tissues.
- Lecithin: cholesterol acyl transferase (LCAT) forms cholesteryl esters from free cholesterol and fatty acids.
- **The exogenous lipid cycle**
- Dietary lipid is absorbed in the small intestine and incorporated into chylomicrons that are secreted into the lymphatics and reach the bloodstream via the thoracic duct. In the circulation, triglyceride is gradually removed from these lipoproteins by the action of lipoprotein lipase. This enzyme is present in the capillaries of a number of tissues, predominantly adipose tissue and skeletal muscle.
- As it loses triglyceride, the chylomicron becomes smaller and deflated, with folds of redundant surface material. These remnants are removed by the liver. The cholesterol may be utilized by the liver to form cell membrane components or bile acids, or may be excreted in the bile. The liver provides the only route by which cholesterol leaves the body in significant amounts.

LIPID METABOLISM

- **The endogenous lipid cycle**
- The liver synthesizes VLDL particles that undergo the same form of delipidation as chylomicrons by the action of lipoprotein lipase.
- This results in the formation of an intermediate density lipoprotein (IDL), which becomes low density lipoprotein (LDL) when further delipidated. LDL may be removed from the circulation by the high affinity LDL receptor or by other scavenger routes that are thought to be important at high LDL levels and the main way in which cholesterol is incorported into atheromatous plaques.
- HDL particles are derived from both liver and gut. They act as cholesteryl ester shuttles, removing the sterol from the peripheral tissues and returning it to the liver. The HDL is taken up either directly by the liver, or indirectly by being transferred to other circulating lipoproteins, which then return it to the liver.
- This process is thought to be anti-atherogenic, and an elevated HDL cholesterol level has been shown to confer a decreased risk of coronary heart disease on an individual.

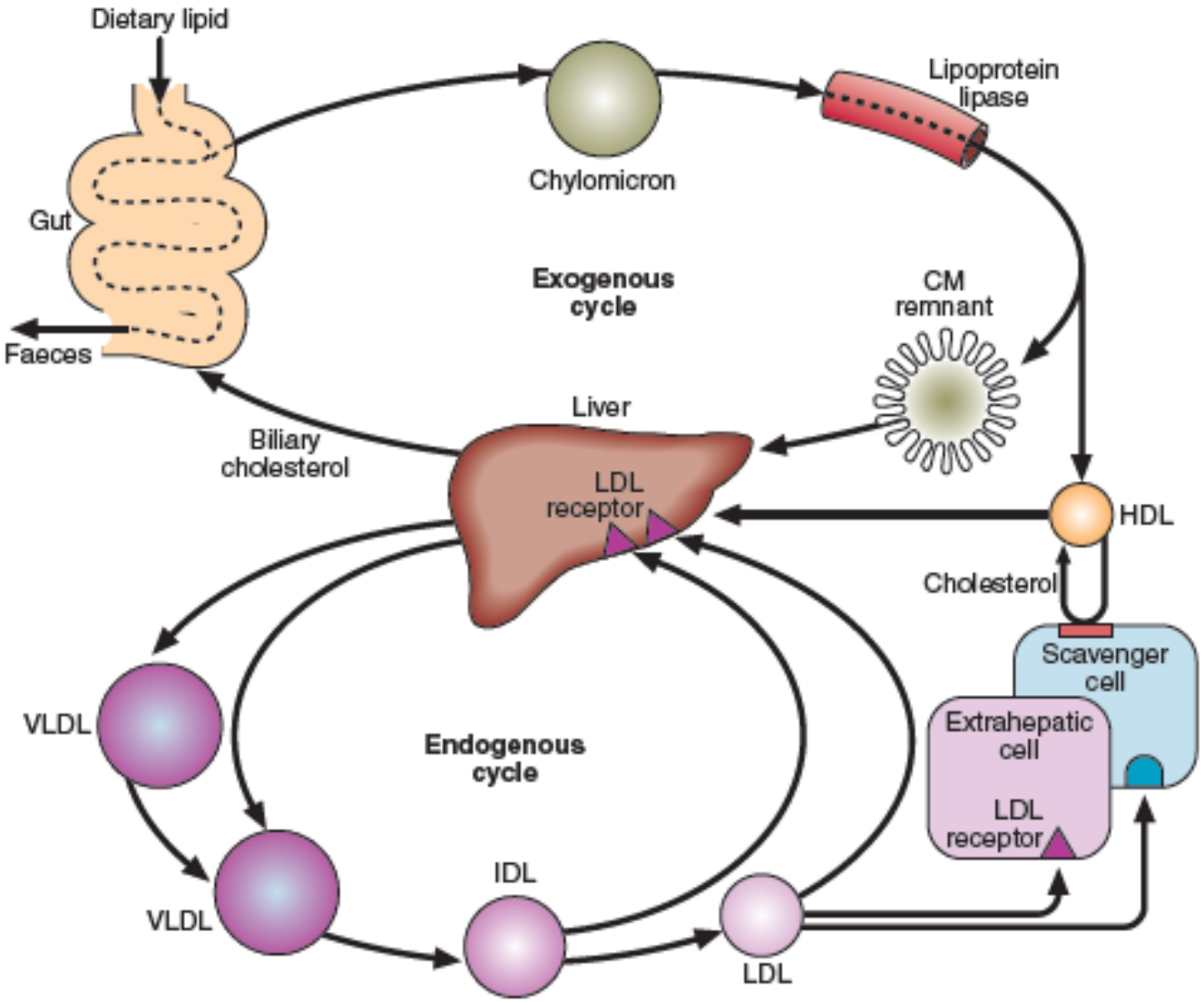
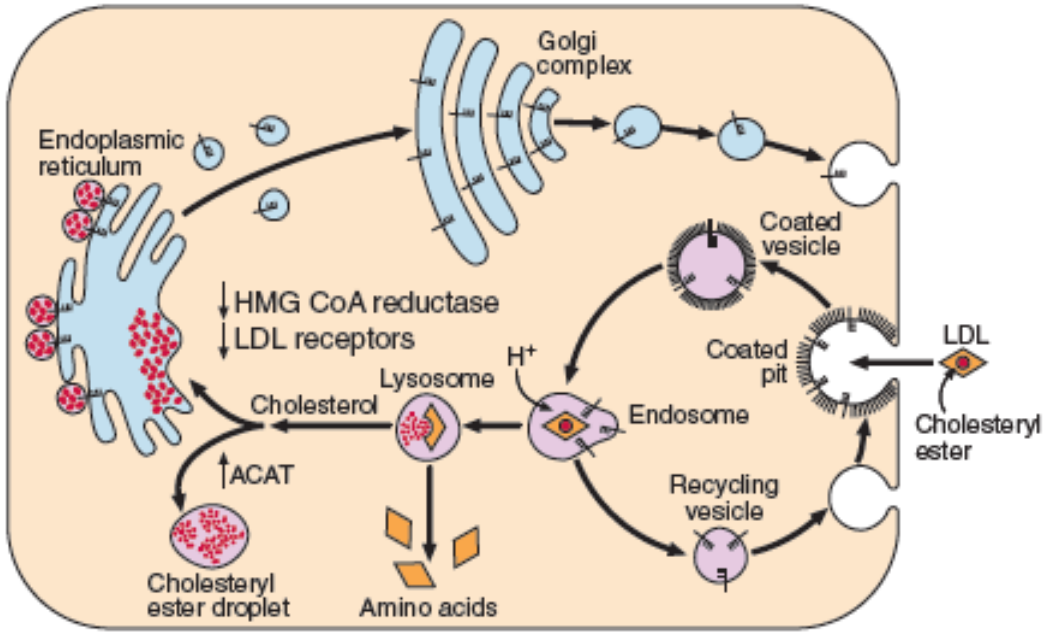


Fig 66.2 **Lipoprotein metabolism.**



HYPERLIPIDEMIA, PRIMARY

- Lipoprotein disorders are some of the commonest metabolic diseases seen in clinical practice. They may present with their various sequelae which include:
- Coronary heart disease (CHD)
- Acute pancreatitis
- Failure to thrive and weakness
- Cataracts.

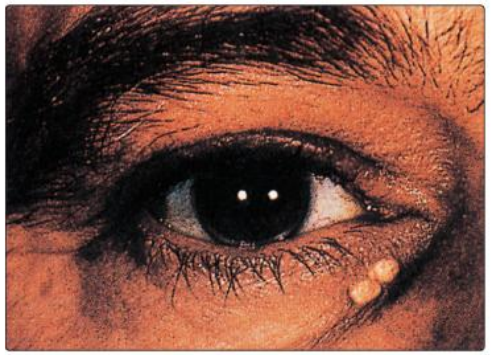


Table 67.1 **Some genetic causes of dyslipidaemia**

Disease	Genetic defect	Fredrickson	Risk
Familial hypercholesterolaemia	Reduced numbers of functional LDL receptors	IIa or IIb	CHD
Familial hypertriglyceridaemia	Possibly single gene defect	IV or V	
Familial combined hyperlipidaemia	Possibly single gene defect	IIa, IIb, IV or V	CHD
Lipoprotein lipase deficiency	Reduced levels of functional LPL	I	Pancreatitis
Apo C-II deficiency	Inability to synthesize apo C-II (cofactor for lipoprotein lipase)	I	Pancreatitis
Abetalipoproteinaemia	Inability to synthesize apo B	Normal	Fat soluble vitamin deficiences, neurological deficit
Analphalipoproteinaemia (Tangier disease)	Inability to synthesize apo A	Normal	Neurological deficit Cholesteryl ester storage in abnormal sites

HYPERLIPIDEMIA, SECONDARY

- Secondary hyperlipidaemia is a well recognized feature of a number of disease that divide broadly into two categories:
- Clinically obvious diseases such as renal failure, nephrotic syndrome and cirrhosis of the liver.
- Covert conditions that may present as hyperlipidaemia. These include hypothyroidism, diabetes mellitus and alcohol abuse.

Table 67.2 **Common causes of secondary hyperlipidaemia**

Disease	Usual dominant lipid abnormality
Diabetes mellitus	Increased triglyceride
Alcohol excess	Increased triglyceride
Chronic renal failure	Increased triglyceride
Drugs, e.g. thiazide diuretics	Increased triglyceride
Hypothyroidism	Increased cholesterol
Nephrotic syndrome	Increased cholesterol

LIPID PROFILE

- The causal association of certain forms of hyperlipidaemia and CHD is clearly the major stimulus for the measurement of plasma lipids and lipoproteins in clinical practice.
- The most common lipid disorder linked with atherogenesis and an increased risk of CHD is an elevated plasma LDL cholesterol level, but increasingly it is being recognized that individuals with low plasma HDL cholesterol and hypertriglyceridaemia are also at increased risk.
- The physical signs of the hyperlipidaemias are not specific for any particular disease and may sometimes be present in normolipidaemic patients, e.g. arcus senilis. Their presence is, however, highly suggestive of raised lipids. Tendon xanthomas are particularly associated with familial hypercholesterolaemia.

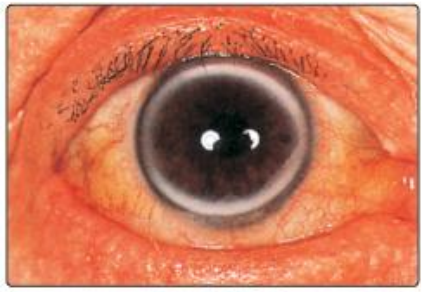


Fig 67.4 **Arcus senilis.**

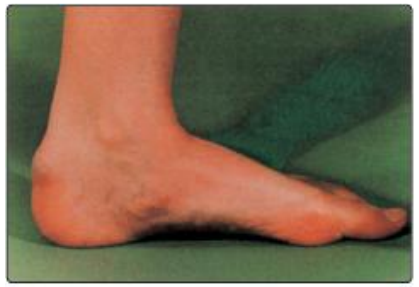


Fig 67.5 **Tendon xanthomas.** These

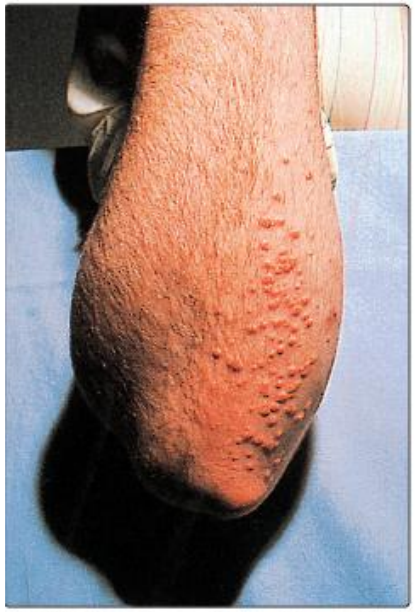


Fig 67.2 **Eruptive xanthomas in a patient with hypertriglyceridaemia.**

TABLE 14-4 CORONARY HEART DISEASE RISK FACTORS DETERMINED BY THE NCEP ADULT TREATMENT PANELS

POSITIVE RISK FACTORS
• Age: ≥45 years for men; ≥55 years or premature menopause for women
• Family history of premature CHD
• Current cigarette smoking
• Hypertension (blood pressure ≥140/90 mm Hg or taking antihypertensive medication)
• LDL cholesterol concentration ≥160 mg/dL (≥4.1 mmol/L), with ≤1 risk factor
• LDL cholesterol concentration ≥130 mg/dL (3.4 mmol/L), with ≥2 risk factors
• LDL cholesterol concentration ≥100 mg/dL (2.6 mmol/L), with CHD or risk equivalent
• HDL cholesterol concentration >40 mg/dL (<1.0 mmol/L)
• Diabetes mellitus = CHD risk equivalent
• Metabolic syndrome (multiple metabolic risk factors)
NEGATIVE RISK FACTORS
• HDL cholesterol concentration ≥60 mg/dL (≥1.6 mmol/L)
• LDL cholesterol <100 mg/dL (<2.6 mmol/L)

TABLE 14-5 TREATMENT GUIDELINES ESTABLISHED BY THE NCEP ADULT TREATMENT PANELS (INITIAL TESTING SHOULD CONSIST OF FASTING FOR ≥12 HOURS)

RISK CATEGORY AND ACTION		
Total cholesterol (TC), <200 mg/dL (5.2 mmol/L); triglycerides (TG), <150 mg/dL (<1.7 mmol/L); LDL cholesterol (LDLC), <130 mg/dL (<3.4 mmol/L); HDL cholesterol (HDLC), ≥40 mg/dL (≥1.0 mmol/L)		
Repeat within 5 years		
Provide risk reduction information		
TC, 200–239 mg/dL (5.2–6.2 mmol/L); TG, 150–199 mg/dL (1.7–2.2 mmol/L); LDLC, 130–159 mg/dL (3.4–4.1 mmol/L); HDLC, ≥40 mg/dL (1.0 mmol/L); and 0–1 risk factors		
Provide therapeutic lifestyle changes (TLC) diet and physical activity information and reevaluate in 1 year		
Provide risk reduction information		
TC, ≥200 mg/dL (5.2–6.2 mmol/L); TG, ≥200 mg/dL (≥2.2 mmol/L); LDLC, 130–159 mg/dL (3.4–4.1 mmol/L); HDLC, <40 mg/dL (1.0 mmol/L); and ≥2 risk factors		
Do clinical evaluation, including family history		
Start dietary therapy (see below)		
TC, ≥240 mg/dL (6.2 mmol/L)		
Perform lipoprotein analysis (see below)		
TREATMENT DECISIONS		
RISK CATEGORY	**ACTION LEVEL**	**GOAL**
DIETARY THERAPY		
No CHD; 0–1 risk factors	≥160 mg/dL (4.1 mmol/L)	<160 mg/dL (4.1 mmol/L)
No CHD; ≥2 risk factors (10-year risk, ≥20%)	≥130 mg/dL (3.4 mmol/L)	<130 mg/dL (3.4 mmol/L)
CHD; CHD risk equivalent (10-year risk, >20%)	>100 mg/dL (2.6 mmol/L)	<100 mg/dL (2.6 mmol/L)
DRUG THERAPY		
No CHD; 0–1 risk factors	≥190 mg/dL (4.9 mmol/L)	<160 mg/dL (4.1 mmol/L)
No CHD; 2 risk factors (10-year risk, ≤10%)	≥160 mg/dL (4.1 mmol/L)	<130 mg/dL (3.4 mmol/L)
No CHD; ≥2 risk factors (10-year risk, 10–20%)	≥130 mg/dL (4.1 mmol/L)	<100 mg/dL (3.4 mmol/L)
CHD; CHD risk equivalent	≥130 mg/dL (3.4 mmol/L)	<100 mg/dL (2.6 mmol/L)

MEASUREMENT OF CHOLESTEROL

- The lipid workup traditionally has begun with measurement of total serum cholesterol. Enzymatic reagents have generally replaced strong acid chemistries in the routine laboratory. Enzymes, selected for specificity to the analyte of interest, provide reasonably accurate quantitation without the necessity for extraction or other pretreatment. Enzymatic reagents are mild compared with the earlier acid reagents and better suited for automated chemistry analyzers.
- The lipoproteins, HDL and LDL, are generally quantified based on their cholesterol content. Thus, the common lipid panel, including measurements of total, LDL, and HDL cholesterol, together with triglycerides, can be completed routinely using chemistry analyzers.

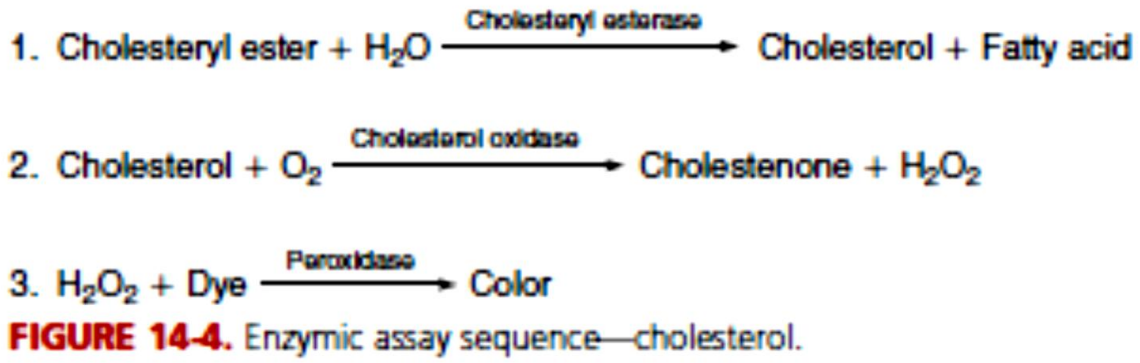


FIGURE 14-4. Enzymic assay sequence—cholesterol.

MEASUREMENT OF TRIGLYCERIDE

- Measurement of serum triglycerides in conjunction with cholesterol is useful in detecting certain genetic and other types of metabolic disorders, as well as in characterizing risk of CVDs. The triglyceride value is also commonly used in the estimation of LDL cholesterol by the Friedewald equation.

1. Triglyceride + H_2O $\xrightarrow{\text{Bacterial lipase}}$ Fatty acid + Glycerol
2. Glycerol + ATP $\xrightarrow{\text{Glycerokinase}}$ Glycerophosphate + ADP
3. Glycerophosphate + O_2 $\xrightarrow{\text{Glycerophosphate oxidase}}$ Dihydroxyacetone + H_2O_2
4. H_2O_2 + Dye $\xrightarrow{\text{Peroxidase}}$ Color

LIPOPROTEIN MEASUREMENT

- Various methods have been used for the separation and quantitation of serum lipoproteins, taking advantage of physical properties, such as density, size, charge, and apolipoprotein content.
- **Electrophoresis** in polyacrylamide gels
- **Chemical precipitation**, usually with polyanions, such as heparin and dextran sulfate, together with divalent cations, such as manganese or magnesium, can be used to separate any of the lipoproteins but are most common for HDL. Apo B in VLDL and LDL is rich in positively charged amino acids, which preferentially form complexes with polyanions. The addition of divalent cations neutralizes the charged groups on the lipoproteins, making them aggregate and become insoluble, resulting in their precipitation leaving HDL in solution.
- **Immunochemical methods**, using antibodies specific to epitopes on the apolipoproteins, have been useful in both research and routine methods. Antibodies have been immobilized on solid supports, such as a column matrix or latex beads.
- **Chromatographic methods**
- **Ultracentrifugation methods:** Preparative ultracentrifugation, uses sequential density adjustments of serum to fractionate major and minor lipoprotein classes.
- Ultracentrifugation is also used in the reference methods for lipoprotein quantitation.

HIGH-DENSITY LIPOPROTEIN METHODS (HDL)

- Because the risk associated with HDL cholesterol is expressed over a relatively small concentration range, accuracy in the measurement is especially important. For routine diagnostic purposes, HDL for many years was separated almost exclusively by chemical precipitation, involving a two-step procedure with manual pretreatment. A precipitation reagent added to serum or plasma aggregated non-HDL lipoproteins, which were sedimented by centrifugation, at forces of approximately 1500*g* (gravity) with lengthy centrifugation times of 10 to 30 minutes or higher forces of 10,000 to 15,000*g*, decreasing centrifugation times to 3 minutes. HDL is then quantified as cholesterol in the supernate, usually by one of the enzymatic assays modified for the lower HDL cholesterol range.
- The earliest common precipitation method used heparin in combination with manganese to precipitate the apo B–containing lipoproteins. Because manganese interfered with enzymatic assays, alternative reagents were developed. Sodium phosphotungstate with magnesium became commonly used, but because of its sensitivity to reaction conditions and greater variability, it was largely replaced by **dextran sulfate** (a synthetic heparin) with magnesium.
- **Polyethylene glycol** also precipitates lipoproteins, but it requires 100-fold higher reagent concentrations with highly viscous reagents, which are difficult to pipet precisely.

HIGH-DENSITY LIPOPROTEIN METHODS (HDL)

- The accepted reference method for HDL cholesterol is a three-step procedure developed at the CDC. This method involves:
  - Ultracentrifugation to remove VLDL,
  - Heparin manganese precipitation from the 1.006 g/mL infranate to remove LDL, and
  - Analysis of supernatant cholesterol by the Abell-Kendall assay.

  - Because this method is tedious and expensive, a simpler, direct precipitation method has been validated by the CDC Network Laboratory Group as a designated comparison method, using direct dextran sulfate (50 kD) precipitation of serum with Abell-Kendall cholesterol analysis

LOW-DENSITY LIPOPROTEIN METHODS

- The most common research method for LDL cholesterol quantitation and the basis for the reference method has been designated betaquantification. Beta-quantification combines ultracentrifugation and chemical precipitation. Ultracentrifugation of serum at the native density of 1.006 g/L is used to float VLDL and any chylomicrons for separation. The fractions are recovered by pipetting after separating the fractions by slicing the tube.
- Ultracentrifugation has been preferred for VLDL separation because other methods, such as precipitation, are not as specific for VLDL and may be subject to interference from chylomicrons.
- In a separate step, chemical precipitation is used to separate HDL from either the whole serum or the infranate obtained from ultracentrifugation. Cholesterol is quantified in serum, in the 1.006 g/mL infranate, and in the HDL supernate by enzymatic or other assay methods.
- LDL cholesterol is calculated as the difference between cholesterol measured in the infranate and in the HDL fraction.

LDL CALCULATION

- A common approach, bypassing ultracentrifugation and commonly used in routine and sometimes research laboratories, is the **Friedewald calculation**.
- HDL cholesterol is quantified either after precipitation or using one of the direct methods, and total cholesterol and triglycerides are measured in the serum.
- VLDL cholesterol is estimated as the triglyceride level divided by 5 (when using mg/dL units), an approximation that works reasonably well in most normolipemic specimens.

- LDL cholesterol = total cholesterol – HDL –Trig/5. This method, commonly performed as the lipid panel, is widely used in estimating LDL cholesterol in routine clinical practice