Dr. Yasir Adil Alabdali

Liver Function Tests

FUNCTIONS OF LIVER

1. **Excretory function:** Liver cells metabolize and excrete endogenous as well as exogenous substances. Liver regulates bilirubin metabolism by secretion and excretion of bilirubin.
2. **Synthetic function:** Synthesis of proteins like albumin, β- and α-globulins, transport proteins and many coagulation proteins occurs in the liver. Liver also produces triglycerides, cholesterol, lipoproteins, and primary bile acids. Albumin maintains osmotic pressure of plasma, transports various compounds, and acts as a protein reserve. **Liver does not synthesize immunoglobulins and complement**.
3. **General metabolic functions:** Liver regulates carbohydrate, lipid, and protein metabolism.

Glycogen, derived from monosaccharides, is stored in the liver. When carbohydrate intake is reduced, blood glucose level is maintained by breakdown of stored glycogen (**glycogenolysis**). If needed, **amino acids** and fat are converted to glucose by the liver (**gluconeogenesis**).
Synthesis of triglycerides, phospholipids, cholesterol, and lipoproteins occurs in the liver. Liver also esterifies cholesterol, and forms **bile acids from cholesterol**. Bile acids are essential for fat absorption from the intestine. Lipoproteins help in transport of fats.
Besides synthesis of various proteins and enzymes, liver is the site for **deamination and transamination** of amino acids. **Ammonia** is converted to **urea** in the urea cycle and detoxified in the liver.

4. Liver is the **storage site** for iron, glycogen, and vitamins.
5. During fetal life, **hematopoiesis** occurs in the liver. It is also a site for destruction of damaged red cells (immune hemolysis).
6. Liver is the major organ for **catabolism of steroid hormones**.

NORMAL BILIRUBIN METABOLISM

Bilirubin is mostly (85%) produced from breakdown of hemoglobin of old red cells in reticuloendothelial cells (macrophages), mainly in spleen. A smaller amount is derived from premature destruction of red cell precursors in bone marrow, and from myoglobin, cytochromes, and peroxidases. Steps in metabolism of bilirubin are outlined below (Fig. 4.2).

1. Hemoglobin is degraded within macrophages to form heme and globin; globin consists of amino acids which are recycled. Heme (iron + protoporphyrin) releases iron, which is stored as ferritin. Protoporphyrin is first converted to biliverdin, which is then reduced to bilirubin.

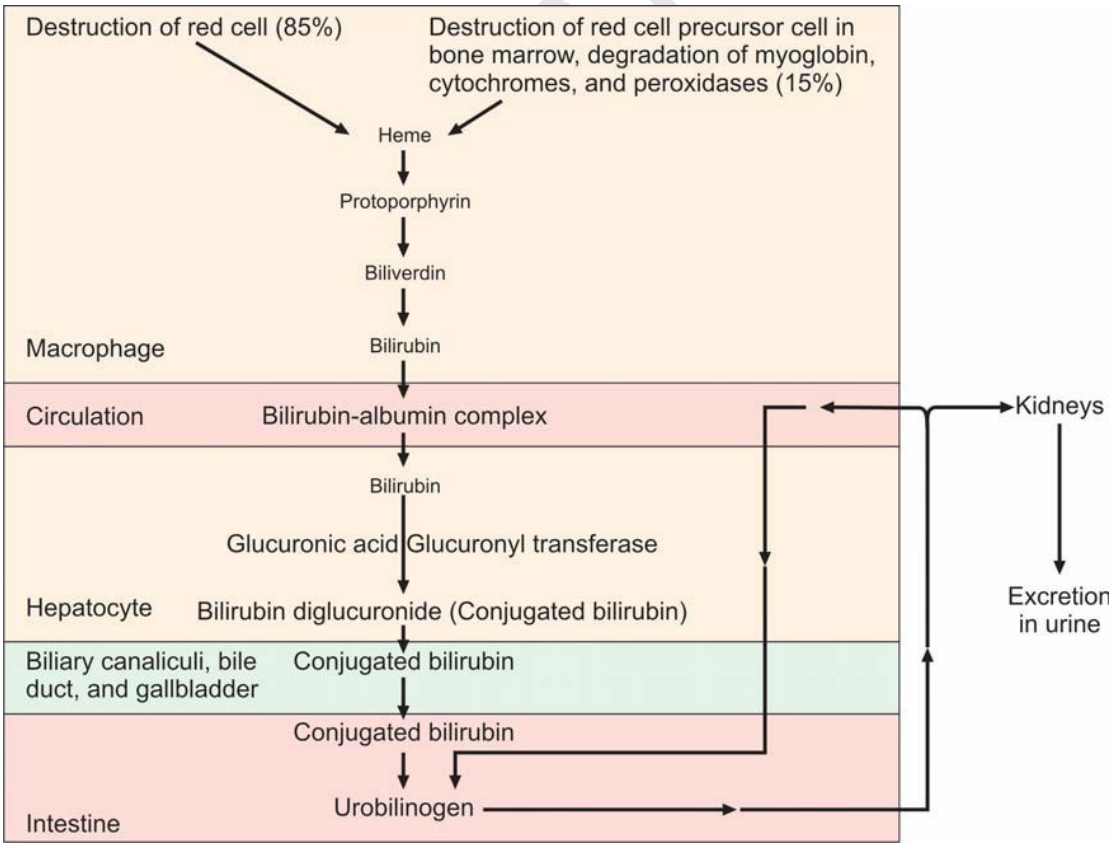
2. Bilirubin is released from macrophages into the circulation where it binds with albumin. This is called as unconjugated bilirubin. It is soluble in lipid but insoluble in water.
3. Bilirubin-albumin complex reaches the liver where it is taken up by the hepatocytes. Bilirubin is set free in the cytoplasm, while albumin is released back into the circulation.

4. Bilirubin is conjugated with glucuronic acid to form bilirubin monoglucuronide and diglucuronide (conjugated bilirubin); this process is mediated by the enzyme glucuronyl transferase. Conjugated bilirubin is more soluble in water.

5. Conjugated bilirubin is secreted from the hepatocyte into the biliary canaliculi, from where it passes into the **bile duct and gallbladder** along with bile (bilirubin monoglucuronide 25% and bilirubin diglucuronide 75%). Bilirubin reaches the small intestine via the common bile duct. Bile also contains bile salts, which are necessary for digestion and absorption of fat from the small intestine.

6. When bilirubin reaches the large intestine, it is converted by bacterial action to a group of compounds known as urobilinogen by the action of bacterial enzymes.

7. Most of the urobilinogen is excreted in feces as **urobilin** and is responsible for **brown coloration of feces**. A part of urobilinogen is absorbed into the circulation from where it reaches the liver, is taken by the hepatocytes, and is again re-excreted in bile (enterohepatic circulation). A small amount of urobilinogen in circulation escapes clearance by the liver and is excreted in urine.



CLASSIFICATION OF LIVER FUNCTION TESTS

Liver function tests can be classified as follows:

1. **Tests that assess excretory function of the liver:** Bilirubin in serum and urine, and urobilinogen in urine and feces.
2. **Tests that assess synthetic and metabolic functions of the liver:** Serum proteins, serum albumin, serum albumin/globulin (A/G) ratio, prothrombin time (PT), and blood ammonia level.
3. **Tests that assess hepatic injury (liver enzyme studies):** Serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase, serum γ-glutamyl transferase (GGT), and 5'-nucleotidase (5'-NT).
4. **Tests that assess clearance of exogenous substances by the liver:** Bromosulphthalein excretion test.

Jaundice

Jaundice (from French jaune, meaning yellow) or icterus refers to yellow discoloration of skin, sclera, and mucous membranes due to increased level of serum bilirubin. Jaundice becomes clinically evident when serum bilirubin level exceeds 2.0 mg/dl.

Classification of jaundice:

1- Prehepatic jaundice (hemolytic jaundice)

In occurs in condition where there is increased destruction of RBCs for instance, in infants with blood group incompatibilities in neonatal physiological jaundice and in hemolytic anemia, megaloblastic anemia and thalassemias. **The liver is basically normal**, so there is an increased formation of **conjugated bilirubin and subsequently of urobilinogen**. This kind of jaundice is characterized by increased free bilirubin in the blood and increased urobilinogen in stool and urine. **Features of haemolysis on blood smear (reticulocytosis, low haptoglobin, low hemoglobin).**

2- Hepatic jaundice

It results from condition that involves the liver cells directly, preventing normal excretion. It can be caused by specific damage such as conjugation failure in neonatal physiological jaundice, where there is an enzyme deficiency. In hepatic disease, **unconjugated, conjugated, or both are increased. Marked rise of serum ALT and AST**

- **Predominantly unconjugated hyperbilirubinemia**
- Gilbert's syndrome
- Crigler-Najjar syndrome
- Physiologic jaundice of newborn
- **Predominantly conjugated hyperbilirubinemia**
- Hepatocellular diseases: viral hepatitis, toxic hepatitis, alcoholic hepatitis, active cirrhosis
- Intrahepatic cholestasis: Dubin-Johnson syndrome, drugs, primary biliary cirrhosis, primary sclerosing cholangitis, biliary atresia and Rotor syndrome

- **Crigler-Najjar syndrome**: This is of two types. Type I is characterized by **autosomal recessive mode of inheritance**, complete absence of **glucuronyl transferase**, severe unconjugated hyperbilirubinemia, and kernicterus (deposition of bilirubin in basal ganglia of brain). Type II is a less severe disease in which some amount of enzyme activity is present.
- **Dubin-Johnson syndrom**: there is a defect in the excretion of bilirubin by hepatocytes, and liver is darkly pigmented due to accumulation of polymerized **epinephrine metabolites**. Bromosulphthalein excretion test is abnormal. In **Rotor syndrome**, there is impaired excretion of bilirubin but without pigmentation of liver; **bromosulphthalein test is normal**. **Alkaline phosphatase is normal in both conditions.**
- **3- Posthepatic or obstructive jaundice**

  This is also called as obstructive jaundice, surgical jaundice, or **extrahepatic cholestasis**. Obstruction of extrahepatic biliary tract prevents flow of bile into the duodenum. This causes "regurgitation" of conjugated bilirubin into the circulation. (Biliary canaliculi distend and rupture due to backpressure of bile and conjugated bilirubin escapes into the sinusoids). Conjugated bilirubin is usually >50% of total in posthepatic jaundice. **Urinary and fecal urobilinogen are decreased**, **faeces are clay-colored, and bilirubin** (being conjugated and water-soluble) appears in urine. The causes of the case are Carcinoma of head of pancreas or Gallstones in or stricture of common bile duct. **Marked rise of serum ALP (>3 times normal) and Abnormal that is corrected with vit K**

Tests which Assess Hepatic Injury (Liver Enzyme Studies)

Serum enzyme changes in liver disease result from hepatocyte damage and do not indicate hepatic functional capacity. In the investigation of liver disease, following serum enzymes are measured:

- Serum aspartate aminotransferase or AST (formerly called serum glutamic-oxaloacetic transaminase or SGOT)
- Serum alanine aminotransferase or ALT (formerly called serum glutamic-pyruvic transaminase or SGPT)
- Serum alkaline phosphatase or ALP
- γ-Glutamyl transferase or GGT (also called as γ-glutamyl transpeptidase)

- **Serum Aminotransferases**

Serum aminotransferases are the sensitive markers of acute hepatocellular injury. ALT is a cytosolic enzyme while AST is both cytosolic and mitochondrial.

Normally, **aminotransferases are present in serum at a low level.** When necrosis or death of cells containing these enzymes occurs, **aminotransferases** are released into the blood and their concentration in **blood increases**. This level correlates with extent of tissue damage. **Normal ratio of both AST/ALT is 0.7 to 1.4.**

Most marked elevations of ALT and AST (>15 times normal) are seen in acute viral hepatitis, toxin-induced hepatocellular damage (e.g. carbon tetrachloride), and centrilobular necrosis due to ischemia (congestive cardiac failure).

Moderate elevations (5-15 times) occur in chronic hepatitis, autoimmune hepatitis, alcoholic hepatitis, acute biliary tract obstruction, and drug-induced hepatitis.
Mild elevations (1-3 times) are seen in cirrhosis, non- alcoholic steatosis, and cholestasis.
Determinations of these enzymes are helpful in the differential diagnosis of hepatocellular from **cholestatic jaundice**. Increase of AST and ALT is much more in hepatocellular jaundice (>500 units/ml) than in cholestatic jaundice (<200 units/ml).

- **Serum γ-glutamyl Transferase (GGT)**

This is also called as γ-glutamyl transpeptidase. Relatively high levels of this enzyme are present in liver, pancreas, kidney, and prostate. Estimation of this enzyme is particularly useful in following liver diseases:
Alcoholism: Increased enzyme activity is present in alcoholism, and is a helpful clue in suspected cases of occult alcoholism (even in the absence of alcoholic liver disease). It is also helpful in follow up of patients with chronic alcoholism. **Marked elevation of GGT occurs in acute alcoholic hepatitis.**
Cholestasis: Elevation of GGT generally parallels that of ALP and 5'-NT in liver disease. **Elevation of ALP is not specific for liver disease. Elevation of both ALP and GGT points towards liver disease.**
Recovery in acute hepatitis: Serum GGT is the last enzyme to return to normal following acute hepatitis and its normalization is indicative of a favourable outcome.

- **5'-nucleotidase (5'-NT)**

5'-NT is present in liver as well as in various other tissues. It is located mainly along the cell membrane, similar to ALP and GGT. Estimation of 5'-NT is helpful in deciding whether increased ALP is **due to liver disease or due to increased osteoblastic activity in growing children.**

- **Serum Alkaline Phosphatase (ALP)**

Alkaline phosphatase is distributed widely in various tissues like liver, bones, intestine, kidney, and placenta. In the liver, ALP, GGT, and 5'-NT are located normally on canalicular surface of hepatocytes.
In cholestasis, accumulated bile acids dissolve canalicular side of hepatocyte membrane and enzymes are released in blood. Therefore, diseases that affect mainly hepatocyte secretion have elevated levels of ALP. Measurement of ALP is helpful in differentiation of hepatocellular jaundice from cholestatic jaundice

Table 4.4: Differentiation of causes of raised aminotransferases

Cause	Clinical features	Diagnosis
1. Viral hepatitis A	H/o exposure in endemic areas	IgM anti-hepatitis A antibodies
2. Viral hepatitis B	H/o nonsterile injections, IV drug abuse, blood transfusion, multiple sexual partners, homosexuals	IgM anti-hepatitis B core antigen antibodies, hepatitis B surface antigen
3. Viral hepatitis C	As for hepatitis B	Hepatitis C-RNA, antihepatitis C antibodies
4. Viral hepatitis E	H/o exposure in endemic area	Anti-HEV
5. Alcoholic liver disease	H/o alcohol abuse	AST/ALT ratio > 2.0, ↑ GGT
6. Nonalcoholic steatohepatitis	Type 2 DM, obesity, hyperlipidemia	Liver biopsy
7. Autoimmune hepatitis	Young female	Raised immunoglobulins, low albumin, antinuclear antibody+, anti-smooth muscle antibody+, liver kidney

			microsomal antibody+
8.	Hemochromatosis	Autosomal recessive, diabetes mellitus, skin pigmentation, chronic liver disease, multiorgan dysfunction	Transferrin saturation >45%, genetic testing
9.	Wilson's disease	Young age, autosomal recessive, Kayser-Fleischer ring, hemolytic anemia	Low ALP, high serum bilirubin, low serum ceruloplasmin

- **DISORDERS OF LIPIDS**

The major lipids present in blood are **cholesterol, fatty acids, and triglycerides**. Lipid disorders are common in clinical practice, and some of them are associated with an increased risk of **atherosclerotic cardiovascular disease**. Cardiovascular disease is a major cause of mortality in persons under the age of 60, and proper management of lipid abnormalities significantly reduces this risk.

Lipids are insoluble in plasma and are therefore transported in circulation in association with proteins. These complexes of lipids and proteins are known as **lipoproteins**. **Dyslipidemias are disorders of lipoprotein metabolism**.

- **PHYSIOLOGY**
- **Cholesterol and Triglycerides**

The two major lipids in blood are cholesterol and triglycerides. Since they are insoluble in water, they are carried by **lipoproteins**.

Cholesterol is a **lipid found in all cell membranes and in blood plasma.** Cholesterol is an **essential component of the cell membranes**, and is necessary for synthesis of **steroid hormones**, and for the formation of **bile acids.** Cholesterol is synthesized by liver and many other organs, and is also ingested in the diet.

Triglycerides are lipids in which three long-chain fatty acids are attached to glycerol. Triglycerides serve as a source of energy. They are present in dietary fat and also synthesized by liver and adipose tissue.

- **Lipoproteins**

Cholesterol and triglycerides are not soluble in water and are transported in blood incorporated into **lipoproteins**. Lipoproteins are spherical macromolecular complexes consisting of (i) a central core of lipids (cholesterol ester, triglycerides, fat-soluble vitamins) that is surrounded by a **surface monolayer** composed of phospholipids, free cholesterol, and apoproteins (Fig. 5.1). The surface of lipoproteins is water-soluble or polar while core is hydrophobic or non-polar.

There **are five major lipoprotein classes: chylomicrons, very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL)** (Fig. 5.2 and Table 5.1). Triglycerides are carried mainly by chylomicrons, VLDL, and LDL, while major lipoproteins for cholesterol transport are LDL and HDL.

Chylomicrons: These are the largest and the least dense of the lipoproteins, and transport **exogenous lipids** to various cells. Dietary fat is incorporated into chylomicrons by intestinal epithelial cells. The lipid core of chylomicrons consists mainly of triglycerides, some cholesterol and fat-soluble vitamins. **Intestinal cells secrete chylomicrons into the lymphatics, which then enter the bloodstream via the thoracic duct**. In circulation, chylomicrons are acted upon by **lipoprotein lipase** to release **triglycerides**; further **hydrolysis of triglycerides** by **lipoprotein lipase** causes

release of **free fatty acids that are then taken up by adipose tissue and muscle**. Liver takes up the cholesterol-rich chylomicron remnant particle and cholesterol enters the metabolic pathway.

Very low-density lipoproteins (VLDL): VLDL particle is synthesized by the liver. It transports triglycerides and cholesterol. **It carries most of the endogenous triglyceride from liver to adipose tissue and muscle.** Triglyceride is removed by the action of lipoprotein lipase in the circulation and VLDL particle becomes smaller, when it is called as **intermediate density lipoprotein (IDL).** Further processing of IDL leads to the formation **of low-density lipoprotein (LDL**), which is the major carrier for **cholesterol.**

Intermediate density lipoprotein (IDL): This is the remnant of VLDL formed when triglycerides are removed from VLDL **by lipoprotein lipase in circulation.**
About half of IDL is cleared from blood by the liver and the remaining half is further processed to form LDL. Normally, IDL is not detected in plasma as it is formed transiently.

Low-density lipoprotein (LDL): LDL is the major carrier lipoprotein for cholesterol from liver to the peripheral tissues. **It is formed from VLDL**. LDL plays a major role in the **genesis of atherosclerosis.**
LDL is taken up by the cells through the LDL receptor, a glycoprotein. The LDL receptor is present on the surface of all cells and recognizes **apolipoprotein B** on the surface of LDL particle. After internalization of LDL particle, the lipoprotein is catabolized and the receptor is recycled back to the cell surface. Intracellularly, LDL is degraded to free cholesterol that is needed for cellular needs.

The level of LDL in circulation is determined by number and function of LDL receptors. Joseph Goldstein and Michael Brown were awarded the Nobel Prize for Physiology or Medicine in 1985 for characterizing **the LDL receptor. Genetic absence of LDL receptors leads to familial hyper- cholesterolemia.**

High-density lipoprotein (HDL): HDL binds to peripheral tissues that have **apolipoprotein A** receptors and takes up cholesterol. HDL cholesterol is either taken by the liver or is incorporated into **IDL to form LDL.**

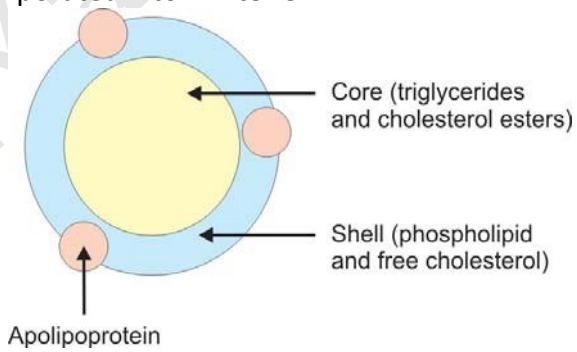
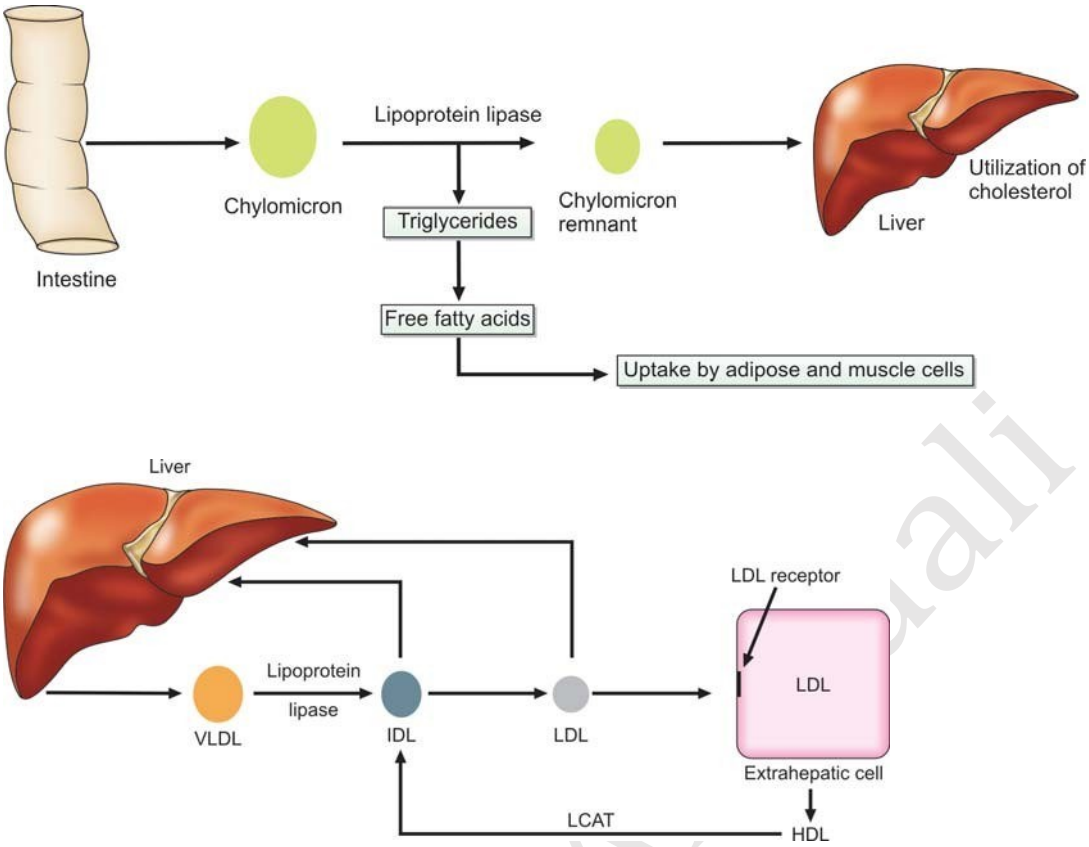


Fig. 5.1: Basic structure of a lipoprotein molecule. Lipoproteins are spherical aggregates of lipids and apolipoproteins. They consist of a core of triglycerides and cholesterol esters surrounded by a shell of phospholipids and cholesterol. Apolipoproteins are embedded in the shell. The larger the lipid core, the lower is its density. Lipoproteins are classified into 5 types: chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL)



Identification of a lipid disorder: Following investigations on a fasting blood sample are usually adequate for identifying lipid abnormalities in majority of cases:

- **Total cholesterol**
- **Triglycerides**
- **HDL-cholesterol**
- **LDL-cholesterol**

Table 5.4: Causes of hypercholesterolemia

Primary	*Secondary*
1. Type IIA and type IIB hyperlipidemia (Familial hypercholesterolemia)	1. Hypothyroidism
2. Common or polygenic hypercholesterolemia	2. Nephrotic syndrome
3. Familial apolipoprotein B100 defect	3. Cholestasis
4. Familial alpha lipoproteinemia	4. Drugs: Protease inhibitors

Table 5.5: Causes of hypertriglyceridemia

Primary	*Secondary*
1. Type I hyperlipidemia	1. Obesity
2. Type V hyperlipidemia	2. Poorly controlled diabetes mellitus
3. Deficiency of lipoprotein lipase	3. Alcoholism
4. Deficiency of apolipoprotein C-II	4. Renal failure
	5. Drugs: Estrogen, glucocorticoids

Acute Coronary Syndrome

The term acute coronary syndrome comprises of conditions characterized by acute **myocardial ischemia and includes** (i) unstable angina, (ii) non-ST segment elevation myocardial infarction (NSTEMI) and (iii) ST segment elevation myocardial infarction (STEMI). The basic pathogenetic mechanism is atherosclerosis of a coronary artery; acute coronary syndrome results from rupture or erosion of an atheromatous plaque with subsequent superimposition of thrombosis (Fig. 5.7).

Cardiac Markers

Myoglobin: Myoglobin is the oxygen-binding low molecular weight protein of **cardiac and skeletal muscle cells**. Myoglobin rises early after myocardial infarction (MI) (1-3 hours) and is currently the earliest marker. Myoglobin of cardiac muscle cannot be distinguished from that of skeletal muscle. Myoglobin levels are raised following MI, open heart surgery, muscle injury, muscle dystrophy, renal failure, shock, and trauma. **Thus, although myoglobin rises early following MI, it is not cardiac-specific.**

Troponins (Tn): Cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are the most sensitive and specific of the available markers of myocardial necrosis and are considered ideal markers for definitive diagnosis (either cTnT or cTnI). Troponins **regulate the interaction of actin and myosin filaments during myocardial contraction**. Following MI, troponins appear in blood at about the same time as Creatine kinase CK-MB. If troponins are elevated at least 12 or more hours following onset of chest pain, their diagnostic sensitivity is 100%.

TnI is more cardio-specific as it is found only in **heart muscle**. It is not elevated following skeletal muscle injury. Following myocardial damage, TnI rises 4-8 hours the onset of chest pain, peaks within 12-24 hours, and remains elevated **for 7-10 days**. **Development of assays for TnI and TnT represent a major advance in the diagnosis of MI**. As troponins remain elevated **for 7-10 days**, they are useful in cases presenting late. If onset of chest pain is 9-12 hours before admission, only troponin needs to be measured.

The most important drugs for the treatment of dyslipidemia

Statins: These drugs inhibit 3- hydroxyl-3-methyglutaryl reductase (**HMG-CoA reductase)**, the rate-limiting enzyme in **cholesterol synthesis**. They are designed to mainly inhibit HMG-CoA reductase in the liver. Inhibition of cholesterol synthesis further decreases circulating LDL because reduced levels of cholesterol in the hepatocyte cause it to upregulate expression of LDL receptors.

PCSK9 inhibitors: PCSK9* is a secreted protein that binds to LDL receptors and targets them for breakdown. PCSK9 inhibitors are monoclonal antibody drugs that bind to PCSK9 and block its ability to cause LDL receptor degradation. They lower LDL cholesterol because with increased numbers of LDL receptors, more LDL can be removed from the circulation.

Ezetimibe

Ezetimibe inhibits cholesterol absorption in the small intestine. This reduces absorption of dietary cholesterol, but also promotes cholesterol excretion, since biliary cholesterol accounts for some of the cholesterol that passes through the small

intestine. Ezetimibe effectively lowers LDL cholesterol, and, when paired with a statin, can provide the same degree of cholesterol lowering with a lower dose of statin. **The most recent results of a large clinical trial testing the combination of ezetimibe with a statin (IMPROVE-IT trial) has shown that it has a modest benefit in reducing heart disease.**

The following beneficial ingredients can be found in Livergenex:

- **Vitamin C** – which boosts energy levels, but also targets liver and gallbladder disease as well as medication toxicity
- **Vitamins B1 & B6** – which target medication toxicity and help improve nonalcoholic fatty liver disease
- **N-Acetyl-L-Cysteine** – typically used to detoxify the liver, support the prevention of alcoholic liver damage, and reduce the toxicity of environmental chemicals such as insecticides
- **Milk Thistle** – a potent herb that is commonly used for chronic inflammatory liver disease, hepatic cirrhosis, chronic hepatitis, and toxic liver damage that is caused by chemical exposure
- **Turmeric** – a powerful herb that targets gallbladder and liver diseases such as fatty liver disease as well as toxic hepatitis
- **Artichoke Leaf** – commonly used for a fatty liver or an alcohol-induced liver injury
- **Resveratrol** – improves fatty liver disease and also helps protect the liver from chemical exposure or alcohol injury
- **Alpha Lipoic Acid** – targets nonalcoholic liver disease and Wilson's Disease