# Cholesterol estimation

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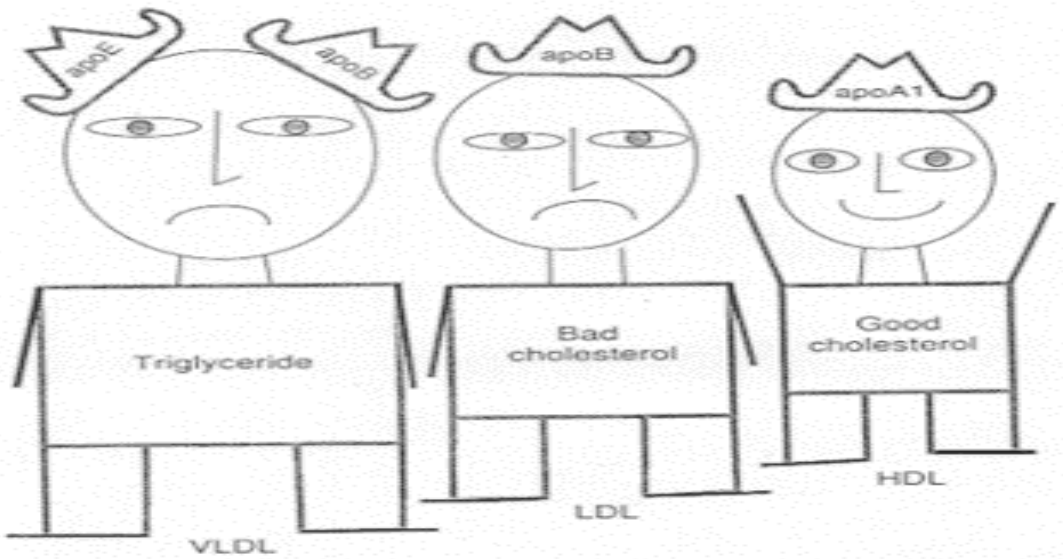
# Objective

- To determine serum cholesterol level

# Intruduction:

**Types of lipids occur in the human body:**

- Cholesterol
- Triacylglycerol




- Polar lipids: Phospholipids and sphingolipids

# Introduction:

- Cholesterol is a member of a large group of substances called steroids, which include vitamin D and a number of steroid hormones, among them the sex hormones of higher animals.

- Cholesterol is an essential component of cell membranes, brain and nerve cells, and bile, which helps the body absorb fats and fat-soluble vitamins.

# Introduction:

- The body uses cholesterol to make vitamin D and various hormones, such as estrogen, testosterone, and cortisol.

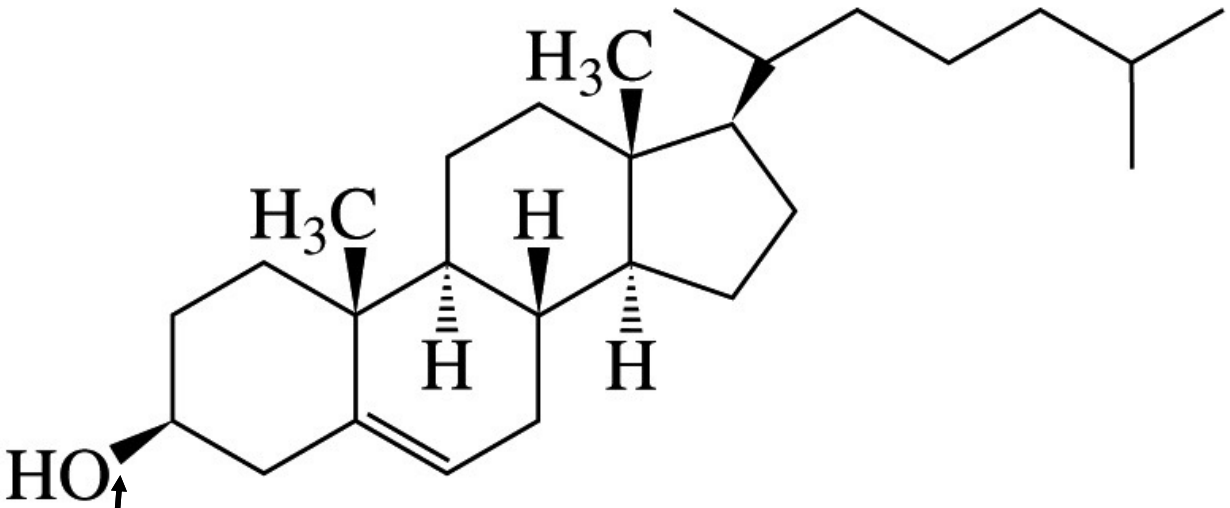
- The body can produce all the cholesterol that it needs, but it also obtains cholesterol from food

# Introduction:

- Cholesterol can be exists in both form esterified and non esterified which depend on the presence of fatty acid at carbon no. 3

- Around 80% of total plasma cholesterol is in the esterified form.

# Cholesterol Structure

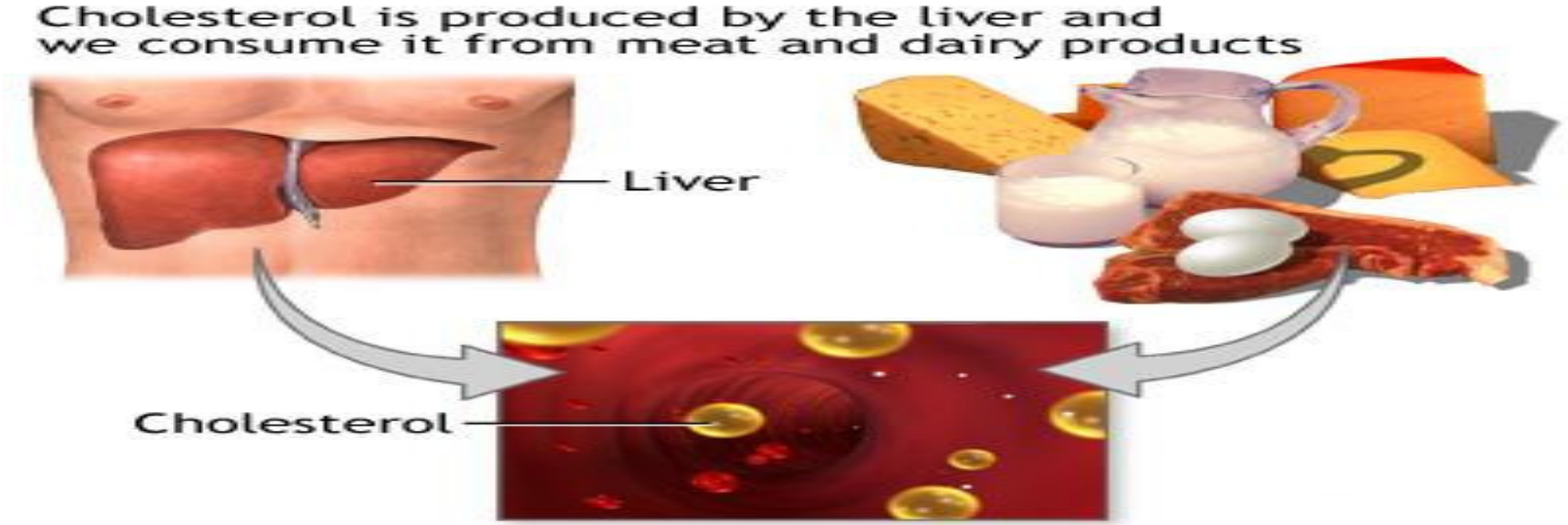



cholesterol

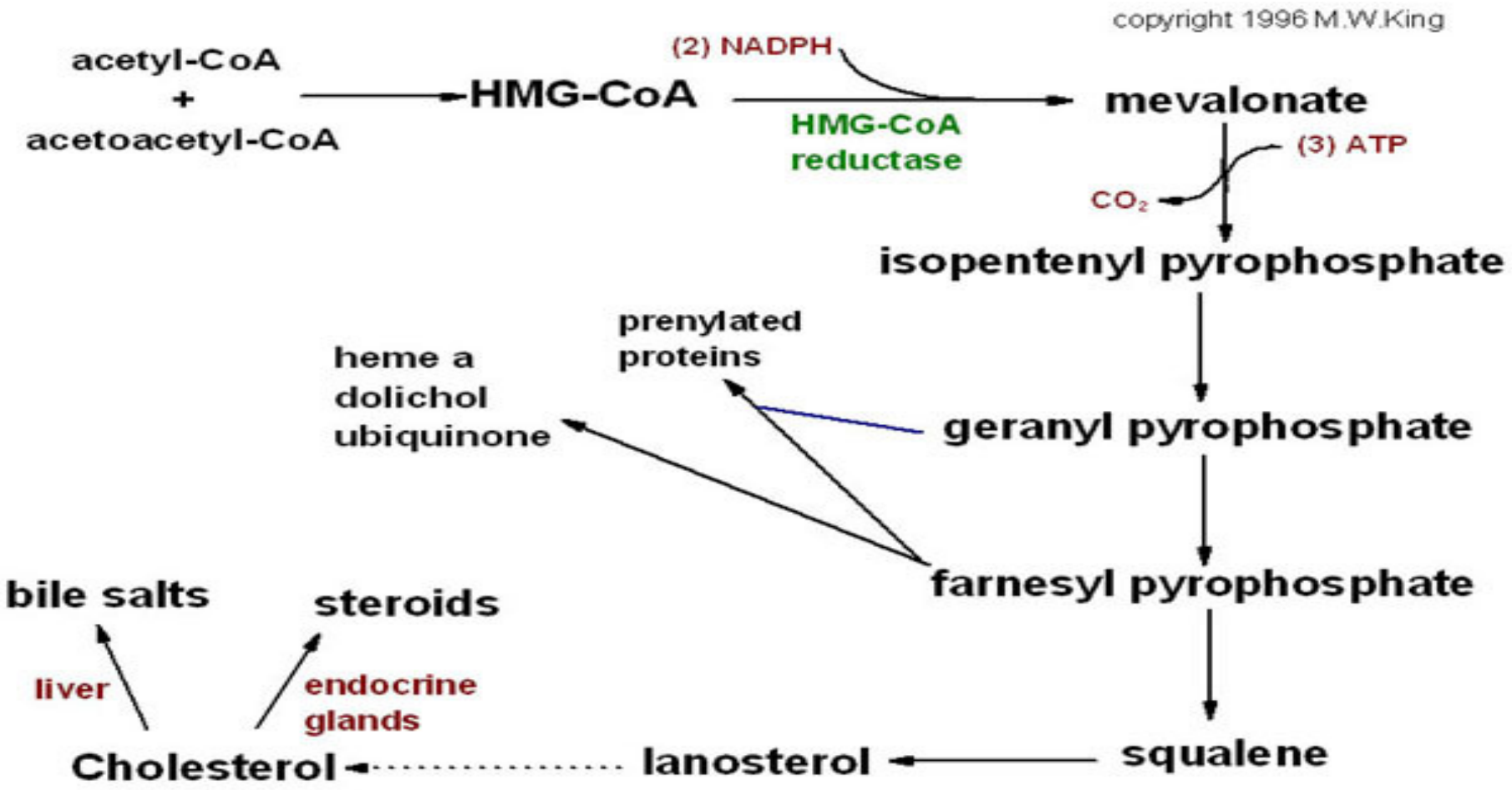
FA for esterification

# Sources of cholesterol

- **Exogenous** : from diet such as egg yolk, red meat and dairy product, it is present in abundance in animal fat
- **Endogenous**: it can be synthesized in liver and intestines



# Biosynthesis of Cholesterol

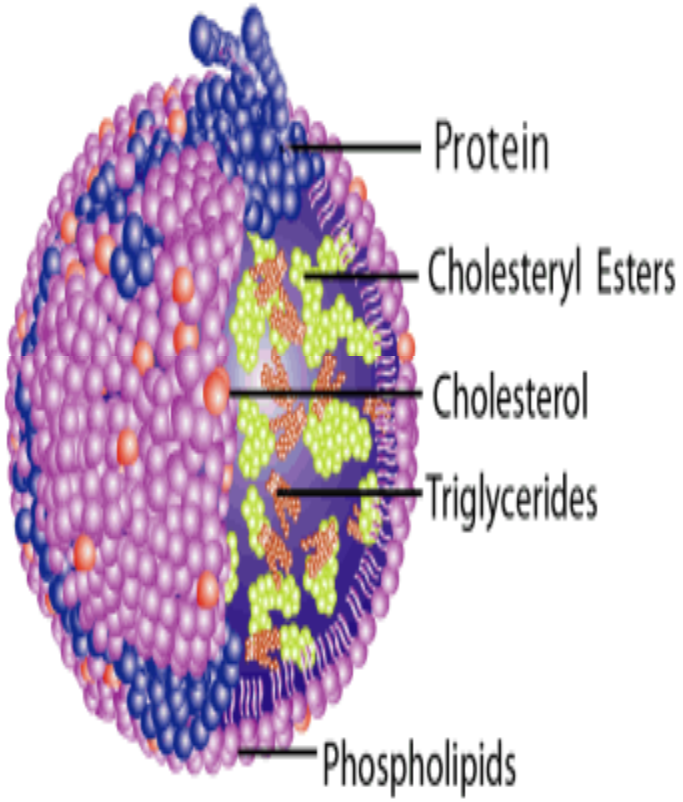



3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)

http://themedicalbiochemistrypage.org/cholesterol.html

# Cholesterol transport:

Fats, such as cholesterol cannot circulate freely in the blood, because blood is mostly water. To be able to circulate in blood, cholesterol packaged with proteins and other substances to form particles called lipoproteins



# There are different types of lipoproteins

Each type has a different purpose and is broken down and excreted in a slightly different way.

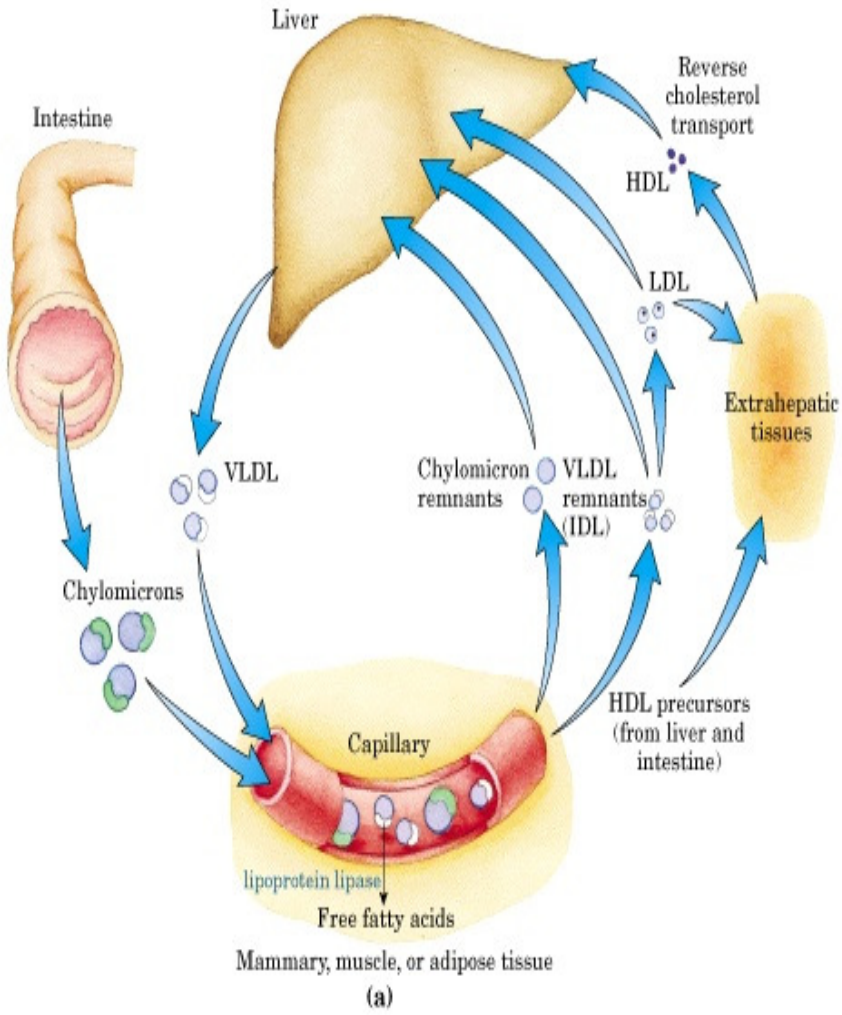
**Lipoproteins include**:

- Very low density lipoproteins (VLDL)
- Low-density lipoproteins (LDL),
- High-density lipoproteins (HDL).

# Low-density lipoproteins (LDL),

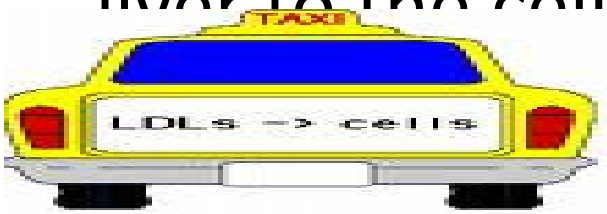
- **LDL** transport 60-70% of cholesterol form liver to cells, it is referred to as bad cholesterol, high level of cholesterol are related to CHD

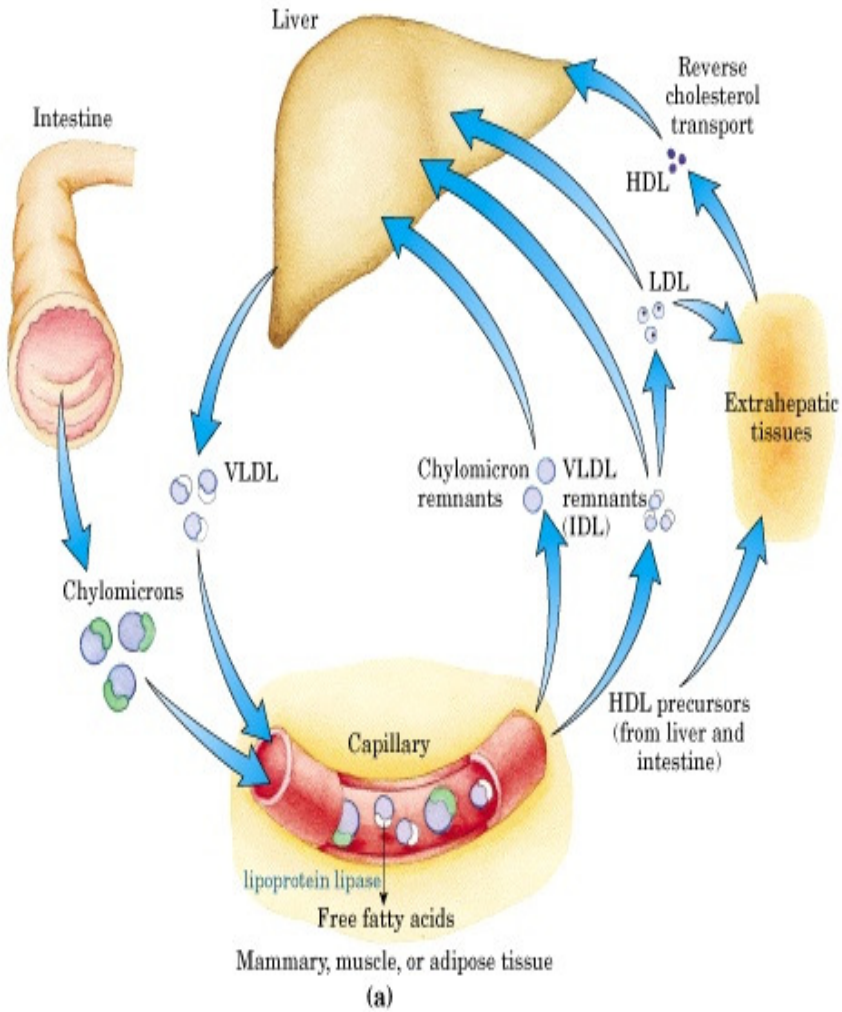
- The major site of cholesterol biosynthesis is the liver.



# Low-density lipoproteins (LDL),

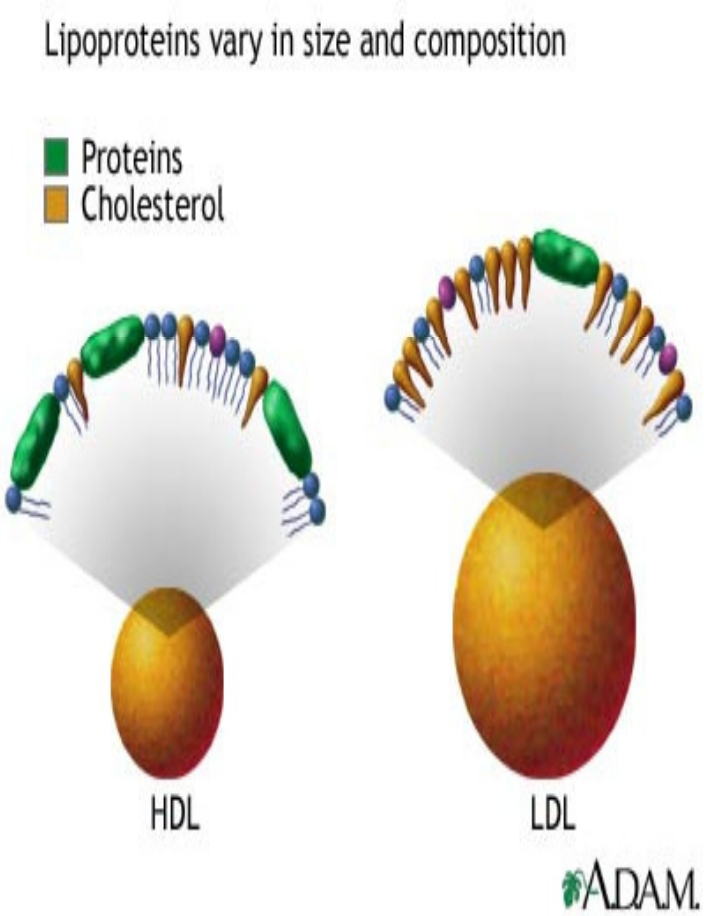
- The major lipoprotein species synthesized by the liver is known as very low density lipoprotein (VLDL), which transports both cholesterol and triacylglycerol from the liver to the cells.



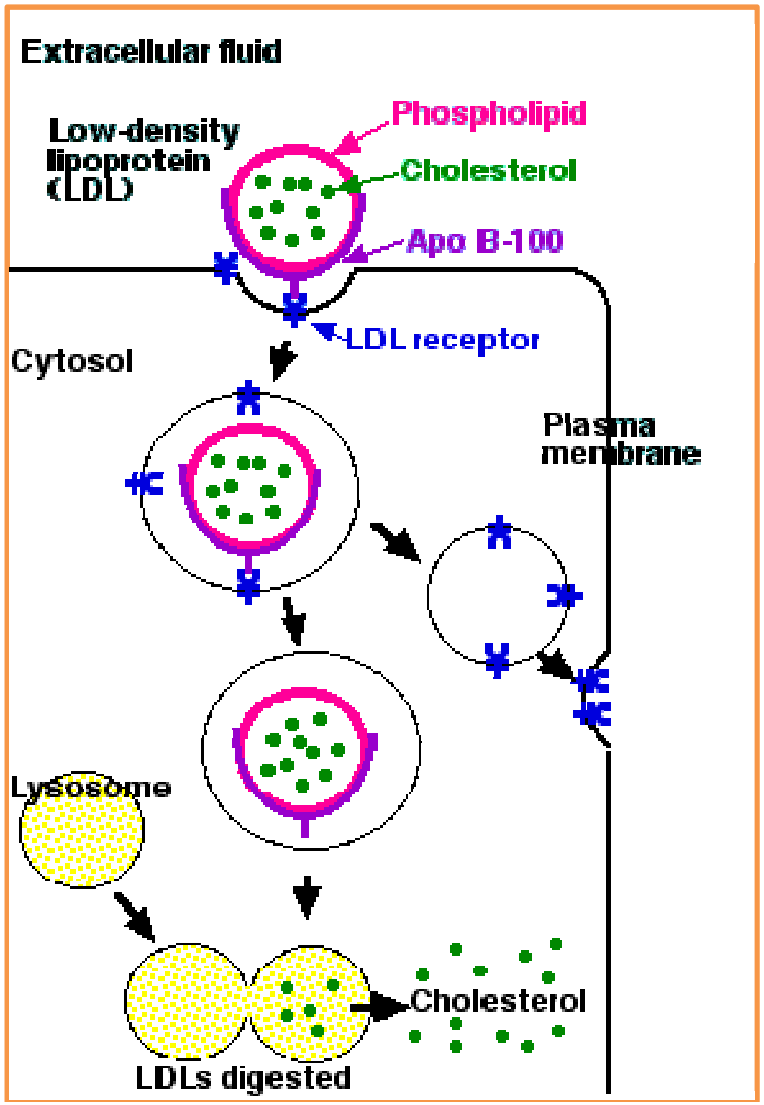
# Low-density lipoproteins (LDL),

- By extraction and exchange of lipids, the composition of VLDL successively changes until it becomes low density lipoprotein (LDL), which has a particularly high concentration of cholesterol.



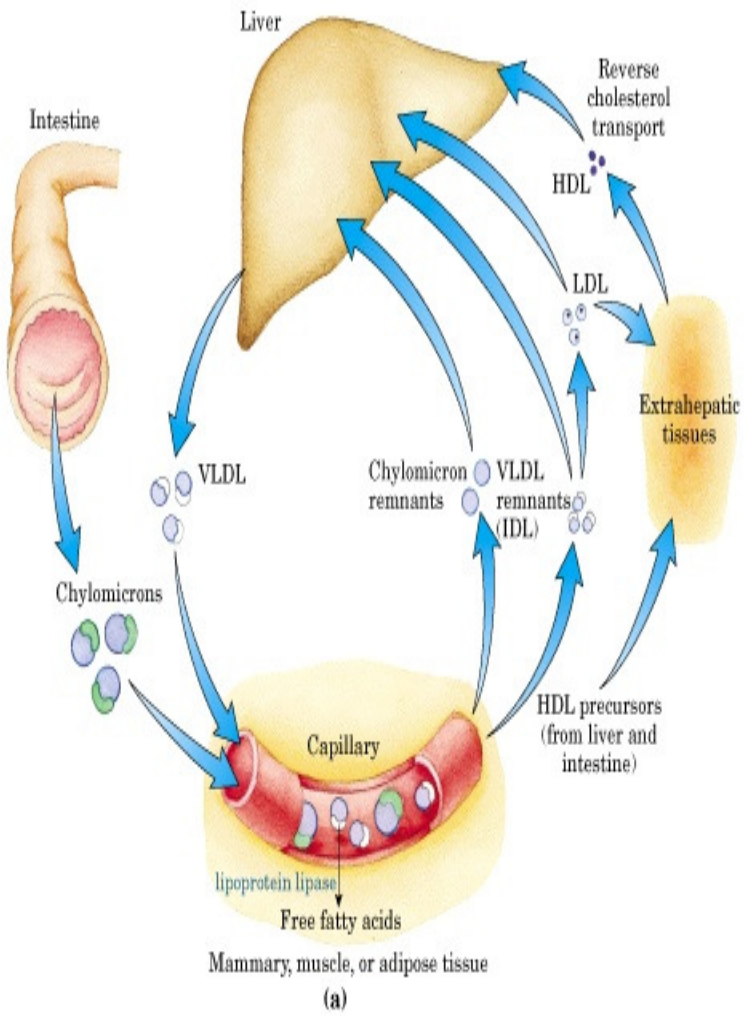
# Low-density lipoproteins (LDL),

- LDL is removed from the circulation through endocytosis by cholesterol-requiring cells, for example in tissues that synthesize steroid hormones. Endocytosis requires a specific receptor, the LDL receptor.



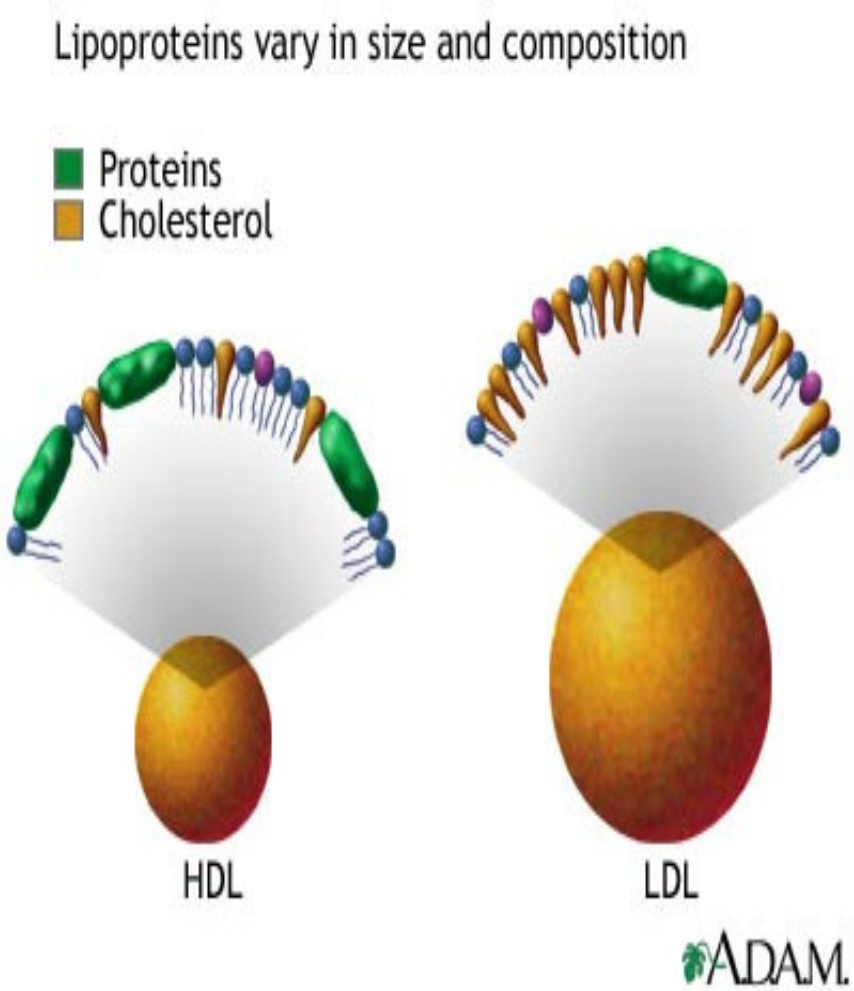
# High-density lipoproteins (HDL)

- High-density lipoproteins (HDL) transport 15-35 % of cholesterol form cells to liver for its excretion and is considered as good cholesterol



# High-density lipoproteins (HDL)

- Excess cholesterol can be transported back from cell tissue to the liver by high density lipoprotein (HDL).
- Among all lipoproteins, this one has the highest ratio of protein to lipid and therefore the highest density.



# High-density lipoproteins (HDL)

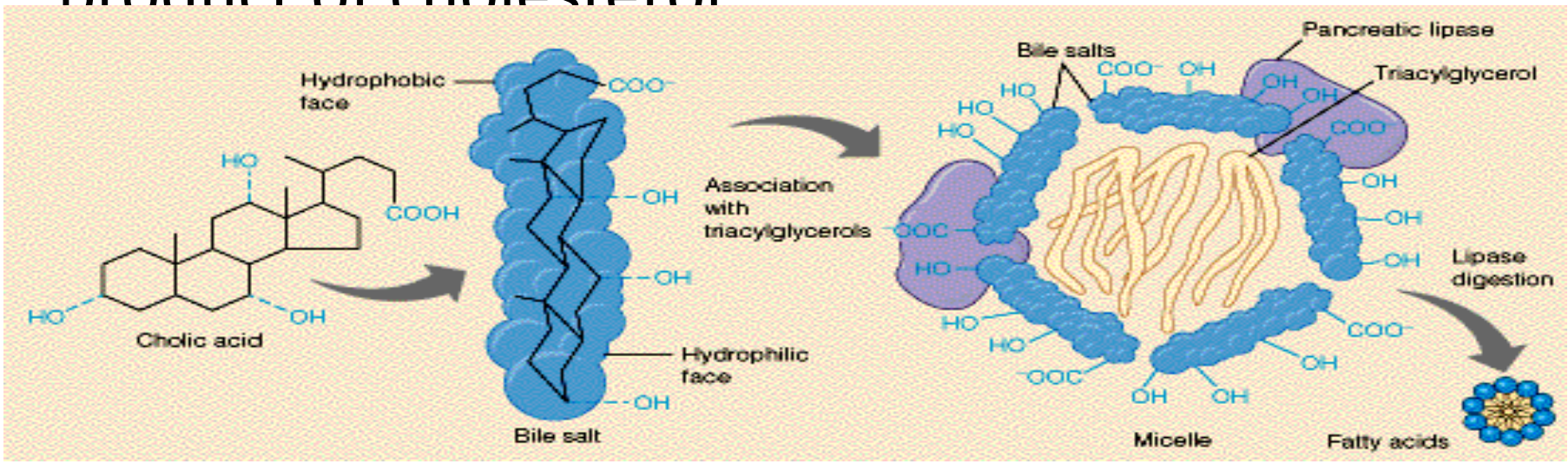
- A high level of HDL in the blood is associated with a *decreased* risk of cardiovascular disease; therefore, HDL has been dubbed the 'good cholesterol' as opposed to LDL, the 'bad cholesterol'.


- The liver can either recycle the cholesterol into new lipoproteins, or dispose of excess cholesterol via the bile (see later).

# Bile acids

- Bile acids are derived from cholesterol and have detergent properties which aid in fat digestion and absorption

- Bile acid synthesis is the major metabolic end product of cholesterol



# Bile acids

- Cholesterol can be eliminated through conversation to salt of bile acid which is performed by the liver, these salt pass to the intestines and excreted as a bile acids

- **Bile acids** are eliminated in the feces but, due to recycling, only about 0.5g/day or less are eliminated in this way

# Cholesterol normal level

- Normal level varies with age, diet and from one location to another, however the average is 150-250 mg/dl

- Cholesterol may elevated slightly in pregnancy

# Low cholesterol

Low cholesterol can be found in certain cases such as:

- Sever infection
- Massive cell damage
- Sever anemia
- Genetic hypolipoproteinemia

# High cholesterol

**High cholesterol can be found in:**

- **Diabetes mellitus** : low insulin level will causes an increase in lipolysis wich in turn increases the amount of free fatty acid in blood. High amount of FFA will lead to increase the amount of TAG formation wich in turn leads to increase in VLDL and LDL synthesis
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# High cholesterol

- **Hypothyroidism** is the disease state in humans and in animals caused by insufficient production of thyroid hormone by the thyroid gland. Thyroxine is important for the transformation of cholesterol to a bile acid

# High cholesterol

- **Obstructive jaundice:** decrease excretion of cholesterol

# Effect of diet on cholesterol level

**Effect of diet:**

- Animal fat increase cholesterol level because they are rich in saturated fatty acid which facilitate esterification of cholesterol

# Effect of diet on cholesterol level

- plant fat decreases cholesterol level because they contain unsaturated fatty acid which blocks the esterification process thus preventing absorption of cholesterol intestinal wall.

# Effect of diet on cholesterol level

- A diet rich in carbohydrate elevates cholesterol level due to the increases of the amount of acetyl COA formed which in turn increases lipids synthesis

# Controlling cholesterol level:

- Restriction of animal fat and carbohydrate in diet
- Increase intake of plants and plant fat
- Drugs should be taken to decrease the level of cholesterol by:

Increasing fecal excretion

Prevent reabsorption

Decreasing VLDL synthesis

# Patient preparation:

- The patient should fast at lest 12 hours before the test
- Water is permitted
- The patient should be on a normal diet at least 7 days before the test
- Lipids lowering drugs such as estrogen, oral contraceptive should be stopped before the test

# Principle of the test

- The reaction depend on the colorimetric determination of a green colored compound resulting from the reaction of cholesterol with acetic anhydride-sulphuric acid (strong dehydrating and oxidizing agent) the absorbance of the green compound is then measured at 610 nm

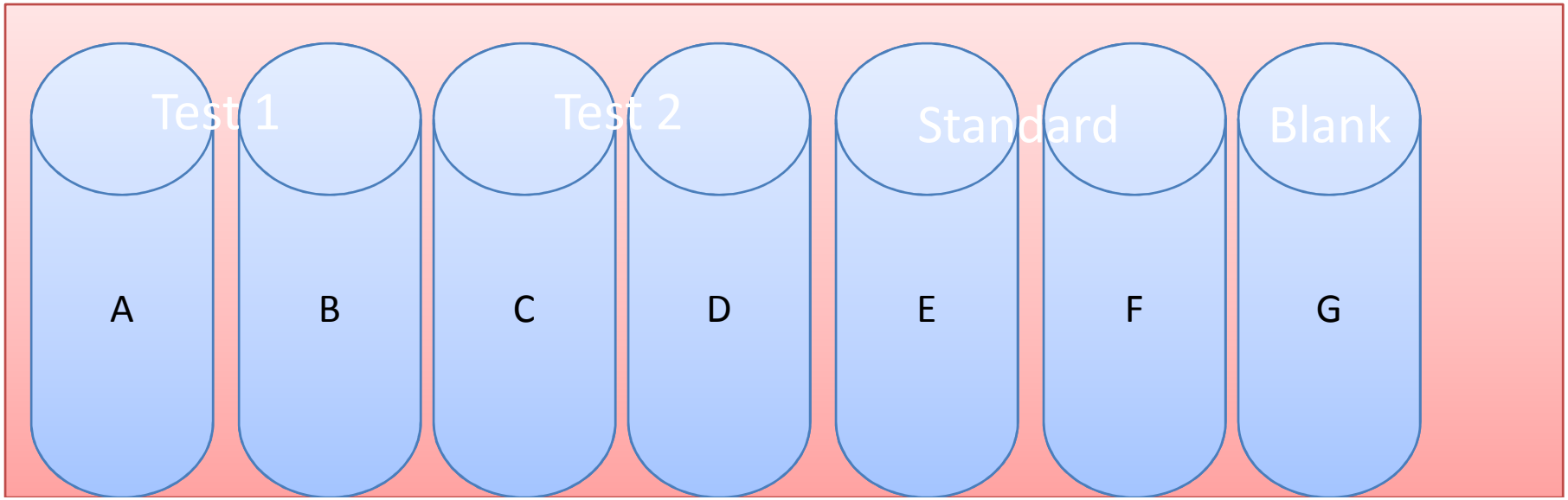
# Material

- Cholesterol reagent (Acetic anhydride + acetic acid
- Sulphuric acid 95-97%
- Standard cholesterol  (300 mgl /dl)
- Samples
- Test tupes
- Pipettes
- Cuvette
- Spectrophotometer
- Water path

# Method

Label 7 test tubes

- Test 1 A B
- Test 2 C D
- Standard E F
- Blank G



# Method

- Pipette the following

| | (A,B) | (C,D) | (E,F) | (G) |
|---|---|---|---|---|
| Sample 1 | 0.1 ml | | | |
| Samble 2 p | | 0.1 ml | | |
| Standard cholesterol | | | 0.1 ml | |
| Distilled Water | | | | 0.1 ml |
| Cholesterol reagent | 4 ml | 4 ml | 4 ml | 4 ml |

# Method

- Stand for 20 minutes
- Pipette 1 ml Sulphuric acid into each tube
- Place the tube in a water bath at room temperature for five minutes
- Remove from the water bath and shake vigorously
- Stand for 10 minutes then measure the absorbance of the sample against the blank at 610 nm

# Result

$$\frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times 300 = \text{---------mg/dl}$$

Comment on your result comparing them with normal values

# Question

- You must make sure that all glass ware used in the experiment is dry. Why?

- Yellow serum is very dangerous. Why?