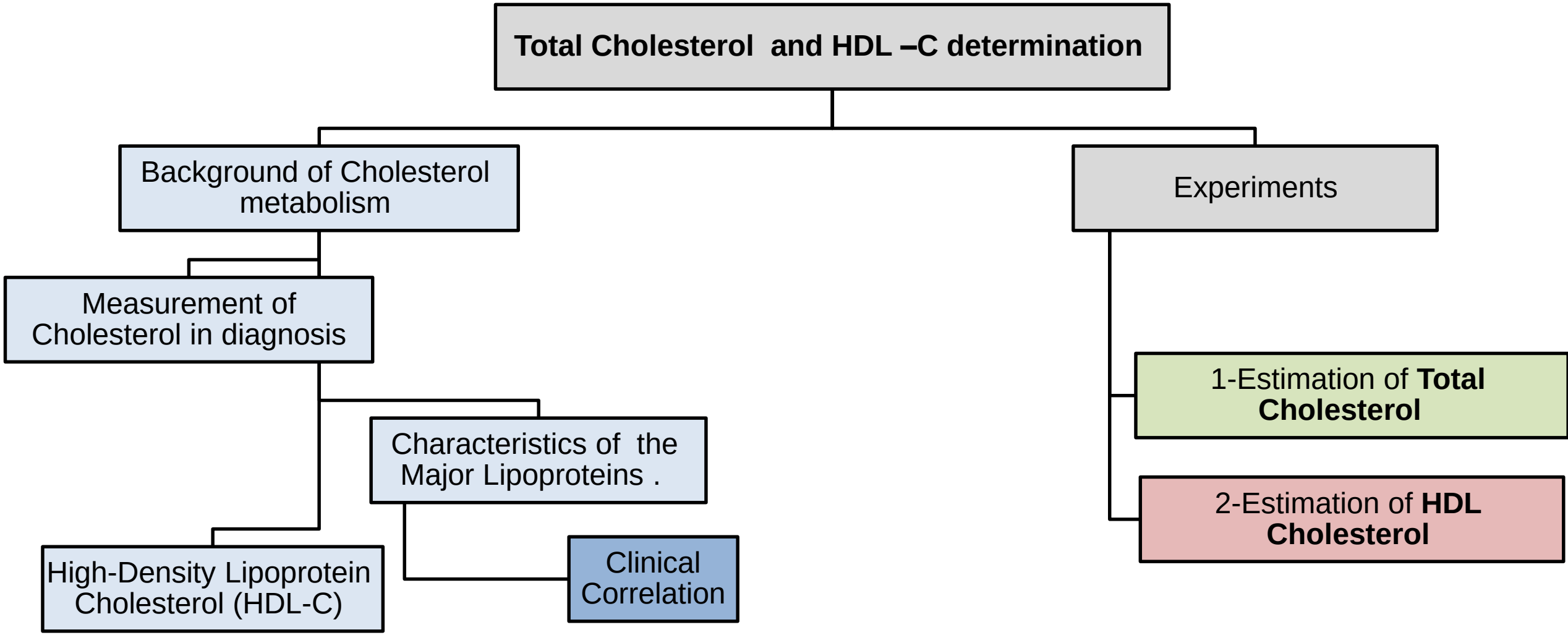
Total Cholesterol determination and HDL-Cholesterol determination

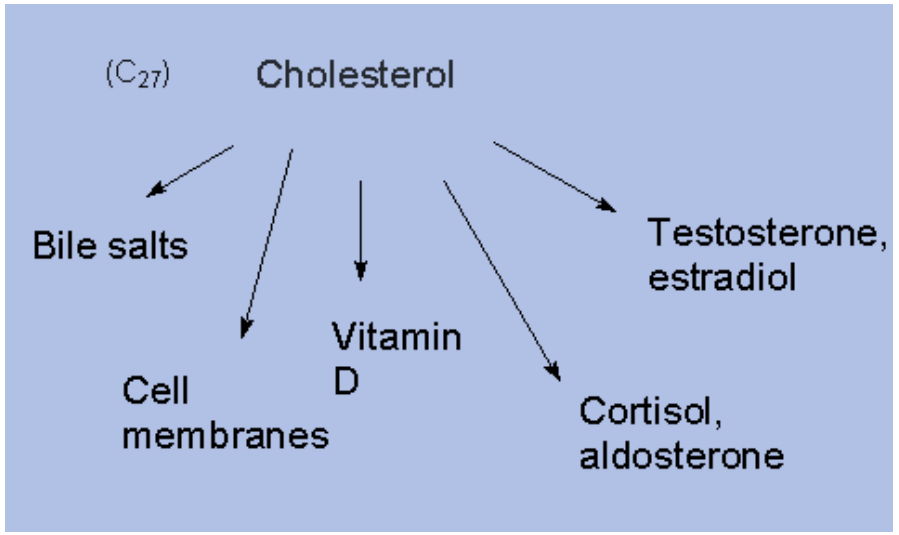
Amal Alamri

Lecture Over view



Background of Cholesterol metabolism

- Although cholesterol synthesized in most tissues of the body where it serve as a component of cell membranes, it is produced mainly in the liver.
- Cholesterol and cholesterol esters are transported in blood lipoproteins.
- Cholesterol is sored in tissues as cholesterol esters .
- In certain endocrine tissues, cholesterol converted to steroid hormone.
- Cholesterol is synthesized endogenously from cytosolic acetyl-CoA



Measurement of Cholesterol in diagnosis

- Cholesterol testing evaluates the risk for: arthrosclerosis, myocardial occlusion, and relates to coronary heart disease (CHD) and it is part of the lipid profiles.
- Elevated cholesterol levels are a major component in the hereditary hyperlipoproteinemias.
- Cholesterol determinations are also frequently a part of :

thyroid function, liver function, renal function, and diabetes mellitus studies. It is also used to monitor effectiveness of diet, medications, lifestyle changes (e.g., exercise), and stress management.

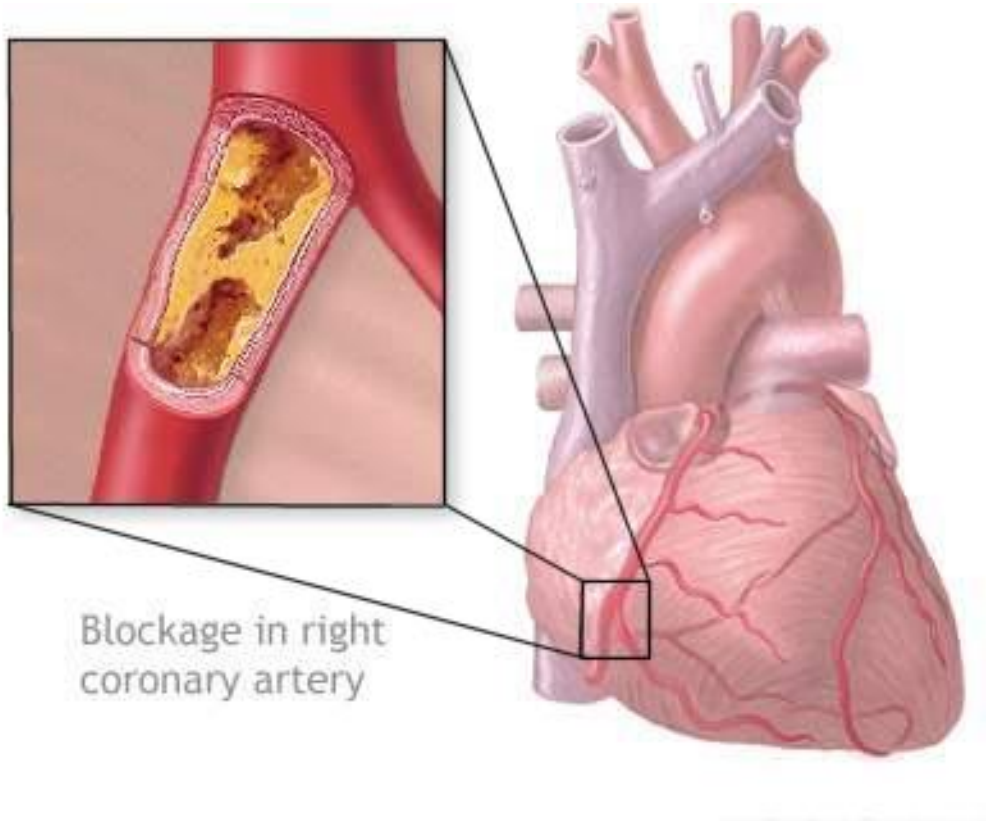
"We found a bunch of these clogging your arteries. They're cholesterol pills."

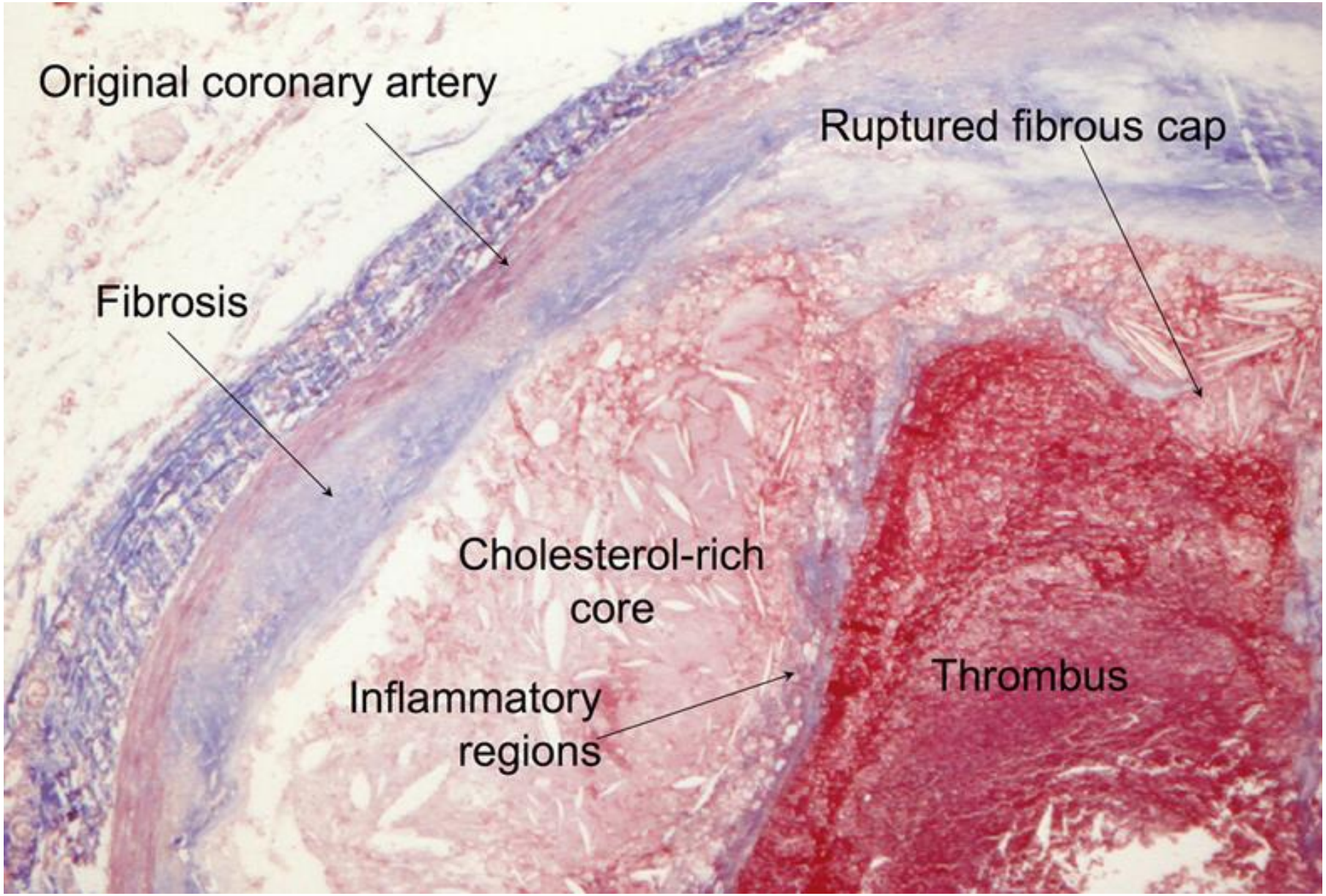
Characteristics of the Major Lipoproteins

Lipoprotein	Density	Particle Diameter	Triglyceride %	Lipid Cholesterol %	Phospho-lipid%	Function
Chylomicrons	0.930	75- 1,200	80-95	2-7	3-9	Deliver dietary lipids
Chylomicrons Remnant	0.930-1.006	30-80				Return dietary lipids to the liver
VLDL	0.930-1.006	30-80	55-80	5-15	10-20	Deliver endogenous lipids
IDL	1.006-1.019	25-35	20-50	20-40	15-25	Return endogenous lipids to the liver, precursor of LDL
LDL	1.019-1.063	18-25	5-15	40-50	20-25	Deliver cholesterol to the cell
HDL	1.036-1.125	9-12	5-10	15-25	20-30	Revers cholesterol transport

CLINICAL CORRELATES

Atherosclerosis involves the formation of lipid-rich plaques in the intima of arteries. The plaques begin as fatty streaks containing foam cells, which initially are macrophages filled with oxidized LDL. These early lesions develop into fibrous plaques that can occlude an artery and cause a myocardial infarct or a cerebral infarct. The formation of these plaques is often associated with abnormalities in plasma lipoprotein metabolism.




Original coronary artery
Ruptured fibrous cap
Fibrosis
Cholesterol-rich core
Inflammatory regions
Thrombus

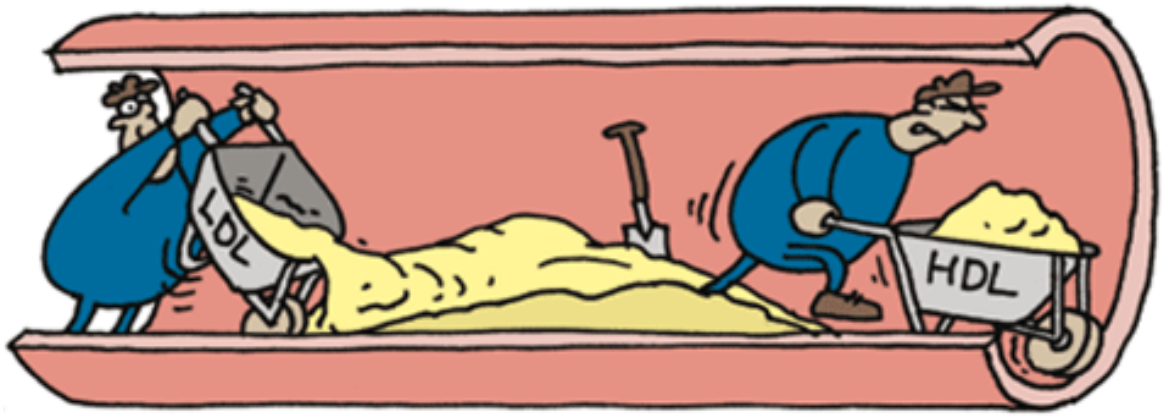
High-Density Lipoprotein Cholesterol (HDL-C)

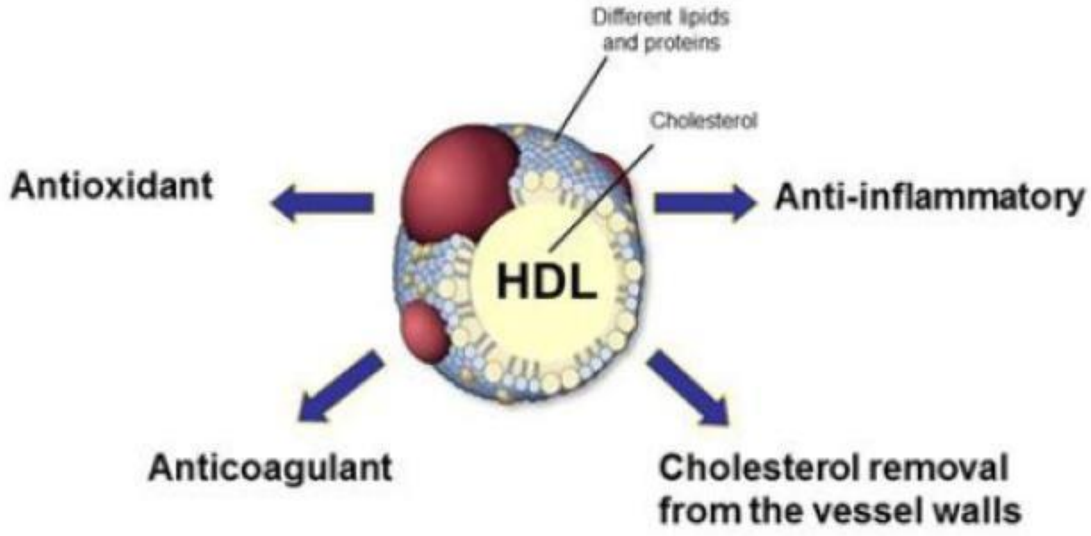
H DL-C is a class of lipoproteins produced by the liver and intestines. HDL is composed of phospho- lipids and one or two apolipoproteins. It plays a role in the metabolism of the other lipoproteins and in cholesterol transport from peripheral tissues to the liver. LDL and HDL may combine to maintain cellular cholesterol balance through the mechanism of LDL moving cholesterol into the arteries and HDL removing it from the arteries.

Function of High-Density Lipoprotein Cholesterol (HDL-C)

Decreased HDL levels are atherogenic, whereas elevated HDL levels protect against arthrosclerosis by removing cholesterol from vessel walls and transporting it to the liver where it is removed from the body.

This is known as the “reverse cholesterol transport path- way.” HDL-C levels are inversely proportional to CHD risk and are a primary independent risk factor.





1-Estimation of Total Cholesterol

Hypercholesterolemia leads to :

- myocardial infarction (heart attack),
- stroke
- peripheral vascular disease.

These balances are can be changed by:

- medications
- food choices.

Hypocholesterolemia

Research into the causes of this state is relatively limited, but some studies suggest a link with:

- depression
- cancer
- cerebral hemorrhage.

In general, the low cholesterol levels seem to be a consequence, rather than a cause, of an underlying illness.

EXPECTED VALUES

RISK CLASSIFICATION	TOTAL CHOLESTEROL (mg/dL)
Desirable	<200
Borderline High	200 - 239
High	≥240

OBJECTIVES

A-Total cholesterol determination in different serum sample.

B.HDL-Cholesterol determination in different serum sample.

"That number has nothing to do with the lottery or the stock market. That's your cholesterol level."

1-Estimation of Total Cholesterol

PRINCIPLE REACTIONS:

The enzymatic reaction sequence employed in the assay of cholesterol is as follows:

Cholesterol Esters $\xrightarrow{\text{Cholesterol ESTERASE}}$ Cholesterol + Fatty Acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol OXIDASE}}$ Cholesten-3-one + H_2O_2

2 H_2O_2 + 4-Aminoantipyrine + Phenol $\xrightarrow{\text{PEROXIDASE}}$ Quinoneimine + $4H_2O$

(Pink dye)

Principle of Total Cholesterol determination :

Cholesterol Esters are hydrolyzed to produce cholesterol, Hydrogen peroxide is then produced from the oxidation of cholesterol by cholesterol oxidase.

In a coupled reaction catalyzed by peroxidase, quinoneimine red colored dye is formed from 4-aminoantipyrine, phenol and hydrogen peroxide.

The absorption of light at 505nm of the solution of this dye is proportional to the concentration of cholesterol in the sample.

MATERIALS

1-CHOLESTEROL (LIQUID) ENZYMATIC REAGENT :

- 4-Aminoantipyrine
- Cholesterol Esterase >150 U/L
- Cholesterol Oxidase
- Peroxidase
- Phenol
- Sodium Cholate
- Non-reactive stabilizers and fillers.

2-CHOLESTEROL STANDARD:

- Cholestrol in alcohol.

3- TOW DIFFERENT SERM SAMPLE

WARNING AND PRECAUTIONS

CAUTION : Cholesterol (Liquid) Enzymatic Reagent contains Phenol. **Avoid contact.**

Specimen should be considered as infectious; handle appropriately.

PROCEDURE

Pipette into clean dry test tubes

	BLANK	STANDARD	Sample 1	Sample 2
Cholesterol (Liquid) Enzymatic Reagent	2.5 ml	2.5 ml	2.5 ml	2.5 ml
Pre-warm at 37 ^{o}C for 3 minutes and add :				
Standard	--	0.025 ml	--	--
Sample 1	--	--	0.025 ml	--
Sample 2	--	--	--	0.025 ml

Mix and incubate at 37 ^{o}C for 10 minutes. Read the absorbance of standard and test at 505 nm against blank.

CALCULATIONS

A = Absorbance)

$$\frac{A(TEST)}{A(Standard)} \times \text{Conc. of Standard} = \text{Conc. of TEST}$$

(mg/dl) (mg/dl)

Concentration of Standard = 200 mg/dl

Calculations of Total cholesterol HDL, LDL and VLDL.

A. Total cholesterol = HDL+ LDL+VLDL.

B.HDL-Cholesterol

C.LDL-Cholesterol can be calculated using the Friedewald formula:

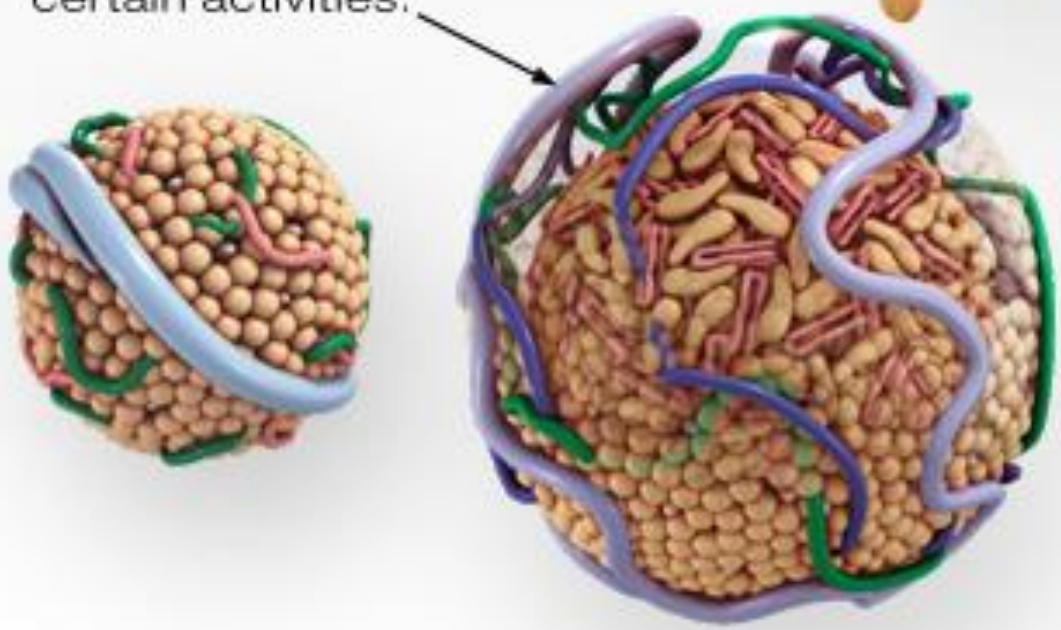
LDL = [total cholesterol] – [HDL+ estimated VLDL].

LDL = total cholesterol – [HDL + (triglycerides÷5)] *Friedewald formula*

- *The estimated VLDL and LDL have more error if triglycerides are above 400 mg/dL. So it can be calculated as*

LDL mg/dL = total cholesterol – HDL –(0.16×triglycerides)

Outer surface proteins provide structure and control certain activities.



Cholesterol esters from the core of the LDL can become plaque in the artery wall.

Triglycerides are a subgroup of lipids found in your blood. They store unused calories that can later be used for energy.

HDL Good cholesterol + **LDL** Bad cholesterol + $\frac{\text{Trigycerides}}{5}$ = **Total cholesterol score**

2-HDL-Cholesterol determination principle :

Principle:

When serum is reacted with the polyethylene glycol reagent, all the low and very low-density lipoproteins (LDL and VLDL) are precipitated. The HDL fraction remains in the supernatant. The supernatant is then used as a sample for cholesterol assay.

HDL-Cholesterol EXPECTED VALUES IN SERUM

HDL-Cholesterol : 33-75 mg/dl

Lower values are associated with an increased risk of coronary heart disease.

(more HDL- cholesterol That indicate low risk to get coronary heart disease.) that is indicated that an inverse relationship exists between serum HDL-Cholesterol and the risk of coronary heart disease.

HDL-Cholesterol determination Procedure

Pipette into clean test tubes/cuvettes:

Chemical	BLANK	STANDARD	Serum Sample
Cholesterol (Liq.)Enzymatic Reagent.	1.0 ml	1.0 ml	1.0 ml
Distilled Water	50 µl	---	---
Standard for HDL (50 mg/dL)	---	50 µl	---
Serum Sample for HDL	---	---	50 µl

- Shaking all tubes and incubate in water bath for 5 min at 37ºC
- Finally; read the absorption at 610 nm .and record your results in the below table

HDL-Cholesterol

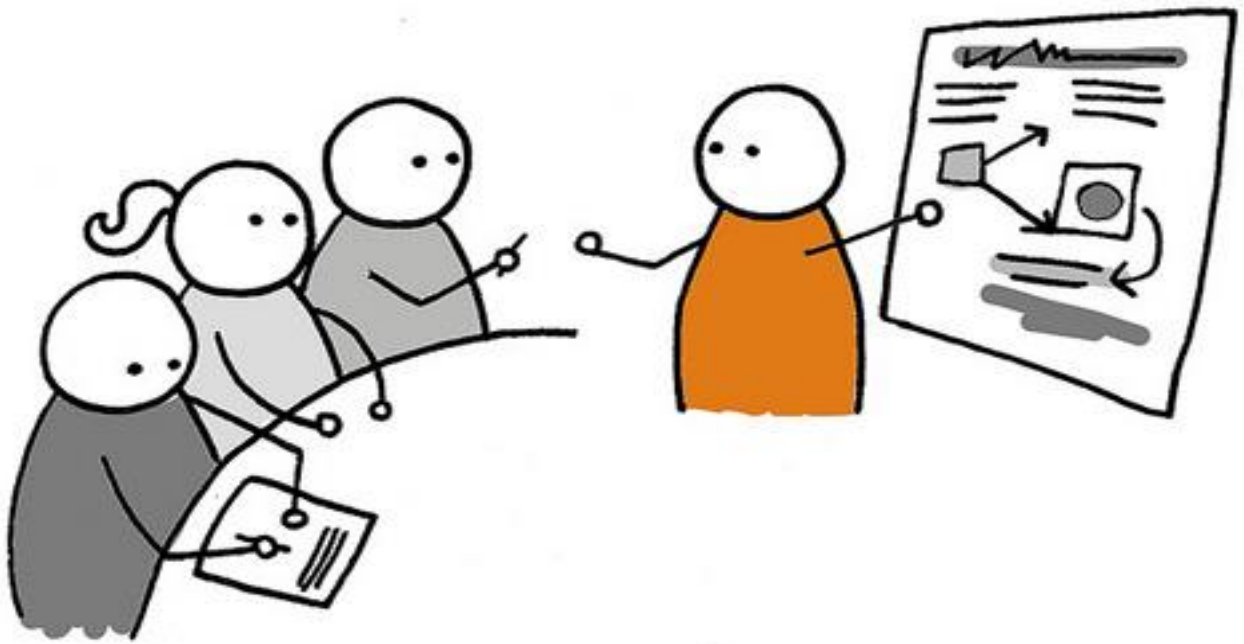
Concentration of HDL- Cholesterol =

$$\frac{\text{Absorbance of the Test}}{\text{Absorbance of the Standard}} \text{ X Concentration of HDLCholesterol Standard} = (300 \text{ mg/dl})$$

DISCUSSION

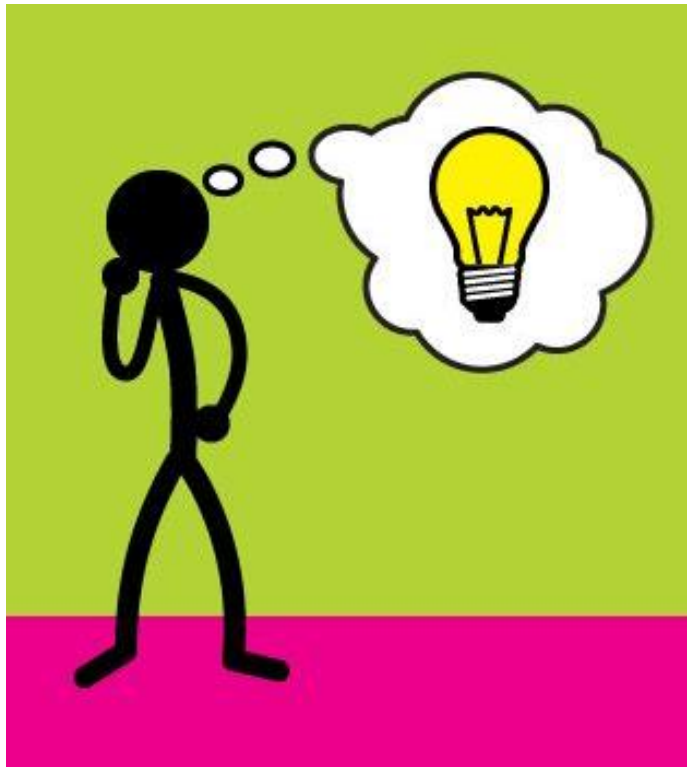
Comment on the level of Total cholesterol in sample 1 and 2 .

Comment on the level of HDL-Cholesterol in the serum sample.



Questions:

- What is the Familial (LCAT) deficiency ? And how it effects the level of cholesterol in blood ?
- What is the Familial (LPL) deficiency?



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