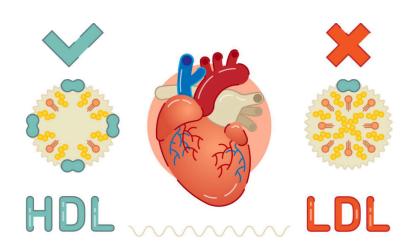
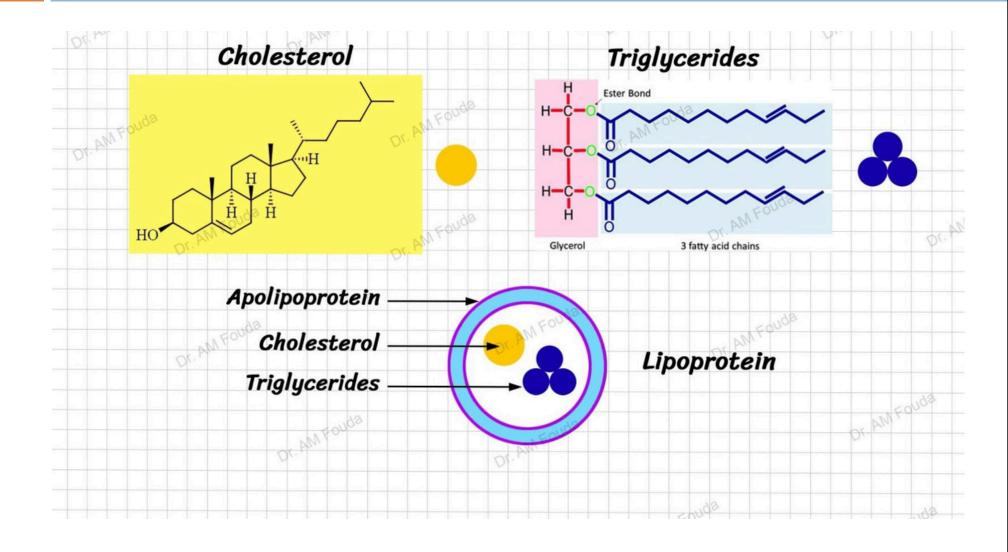




LIPIDSPROFILE



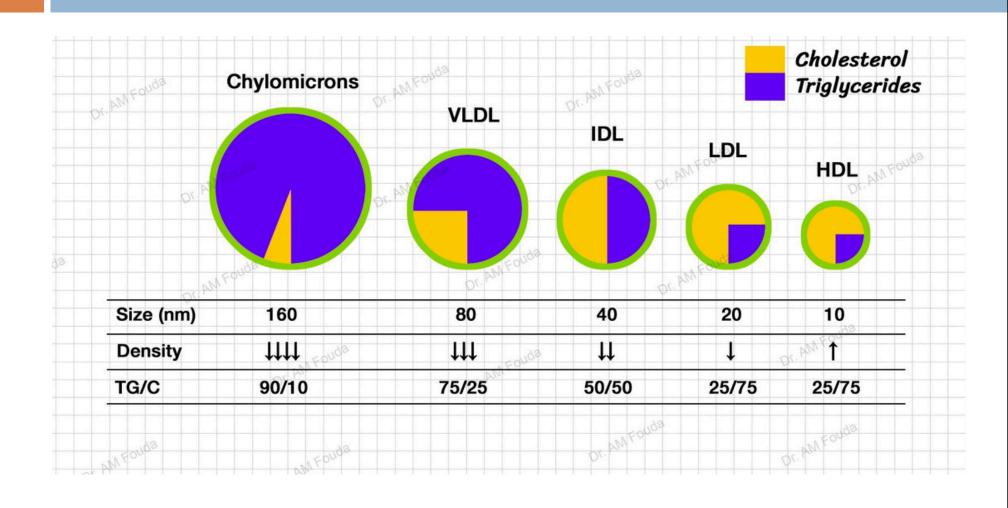
Basic information



Blood lipoprotein

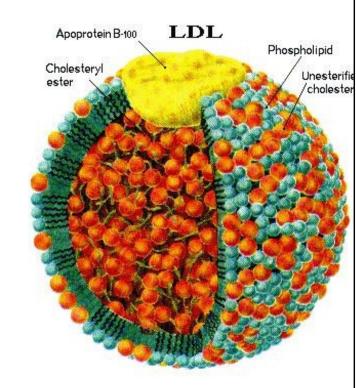
- Composed of: Cholesterol, Cholesterol ester, TG, Phospholipids and Protein
- Four major types: vLDL, LDL, HDL and Chylomicron
- They differ in the contents of each composition.
- Function: transport lipids in blood to organs (lipids are hydrophobic and can't transport in blood without carrier)
- Then these lipids are either: stored in adipose tissue or oxidized to give energy

Types of Lipoproteins



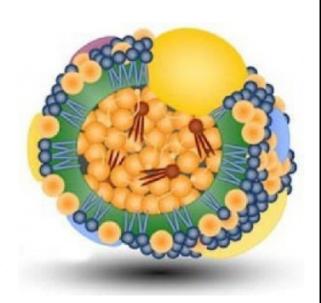
LDL (low density lipoprotein)

- LDL: bad cholesterol "carry cholesterol from liver to blood then to organs
- It has less protein content and contains more cholesterol.
- LDL cholesterol is easy to stick to the walls of blood vessels.
- High LDL in blood associated with atherosclerosis, heart disease and myocardial infraction
- Reducing LDL levels is a major treatment target for cholesterol-lowering medications.
- Because high LDL in blood will deposited in blood artery and trigger clot formation

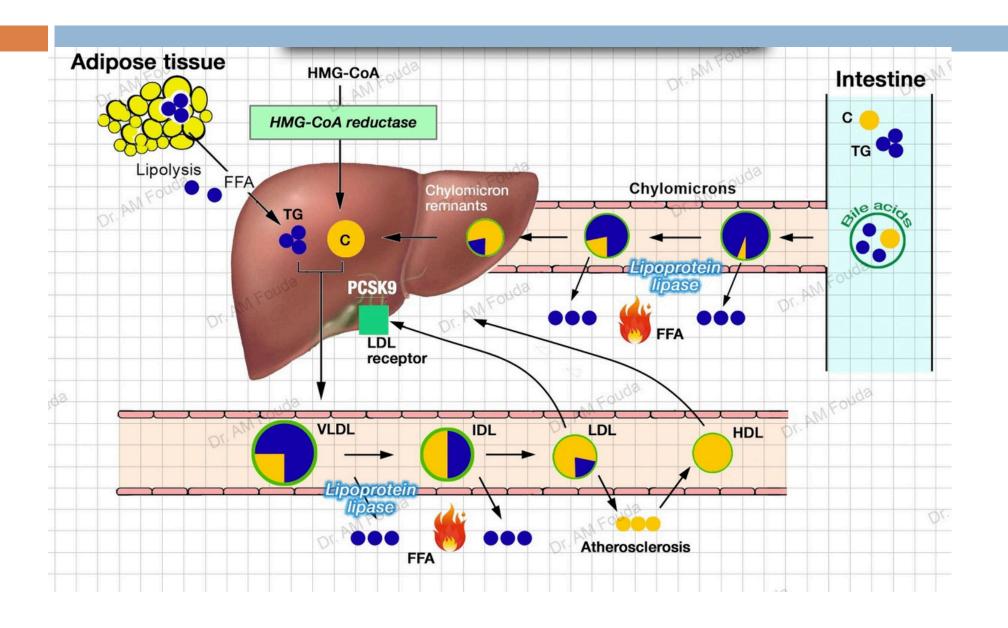


HDL (high density lipoprotein)

- HDL: good cholesterol, carry cholesterol from organs and blood to liver to get rid of it
- It removes excess cholesterol from tissues (it cleans blood).
- High levels linked to a reduced risk of heart and blood vessel disease. The higher your HDL level, the better.



Lipoprotein metabolism



Hyperlipidemia

• Is the condition of abnormally elevated levels of any or all <u>lipids</u> and/or <u>lipoproteins</u> in the <u>blood</u>. It consider a heterogeneous group of disorders.

Hyperlipidemia

- lipoproteins
- cholesterol
- triglyceride

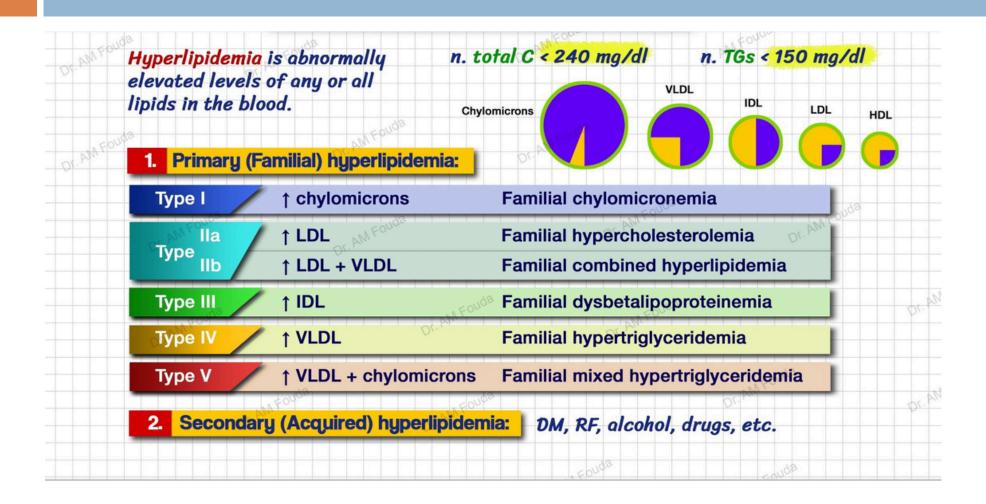
Hyperlipidemia: elevated levels of lipids, i.e. triglycerides and cholesterol in blood

Hyperlipoproteinemia: elevated levels of lipoproteins, especially VLDL, IDL and LDL

Hypercholesterolemia: elevated levels of cholesterol in blood

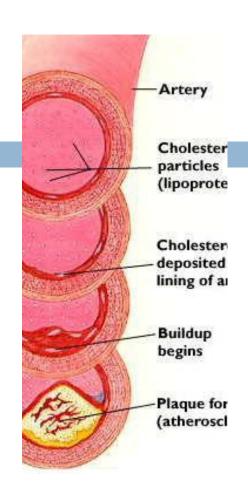
Hypertriglyceridemia: elevated levels of triglycerides in blood

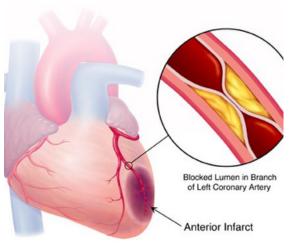
Classification of Hyperlipidemia



Risk of high LDL and heart disease

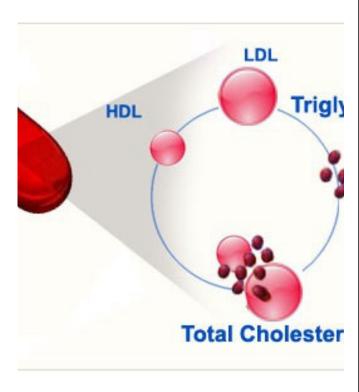
- High blood LDL will deposit cholesterol in the inner walls of the arteries that feed the heart and brain.
- It can form plaque (thick, hard deposit) that can narrow the arteries and make them less flexible.
- This condition is known as atherosclerosis.
- If a clot forms and blocks a narrowed artery, heart attack or stroke can result.





Lipids profile

- Lipid profile: A pattern of lipids in the blood. A lipid profile usually includes the levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and Using these values, a laboratory may also calculate: Very low-density lipoprotein and Cholesterol: HDL ratio.
- It requires a 12-hour fast (no food or drink, except water). For the most accurate results, wait at least two months after a heart attack, surgery, infection, injury or pregnancy to check lipid levels.



Measuring TG levels

Principle of determination

$$\begin{array}{c} \text{lipoprotein lipase} \\ \text{Triglyceride} & \longrightarrow & \text{glycerol + fatty acids} \\ \\ & & \text{glycerol kinase} \\ \text{Glycerol + ATP} & \longrightarrow & \text{glycerol-3-phosphate + ADP} \\ & & \text{Mg}^{2+} \\ & & \text{Glycerol-3-phosphate-oxidase} \\ \text{Glycerol-3-phosphate + O}_2 & \longrightarrow & \text{dihydroxi-acetone phosphate + H}_2\text{O}_2 \\ \\ & & \text{peroxidase} \\ \text{H}_2\text{O}_2 + \text{amino-4-antipyrine + ESPAS} & \longrightarrow & \text{red derivative of quinone+ 4 H}_2\text{O} \\ \end{array}$$

Experiment:

Prepare the following tubes:

Solution / #	1	2	3	4
Reagent 1+2.	1 ml	1 ml	1 ml	1 ml
TAG Standard (Ψ.	20 μΙ		-
Sample (normal)		-	20 μΙ	-
Sample(ill)	-			20 μΙ
Distilled water)	20 μΙ		-	-

Shake the tubes well, incubate them for 5 min. at 37°C. Read the optical density of the samples (A) at 505 nm. Tube#1 serves as blank.

Calculation:

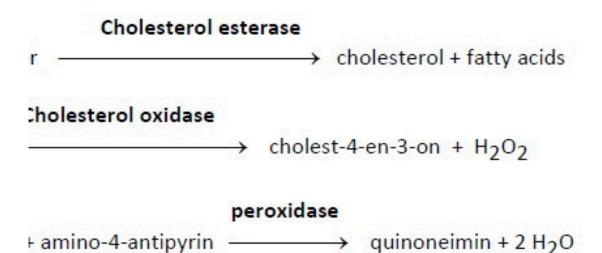
The concentration of triglycerides present in the samples can be calculated from the measured absorbance of the samples and of the standard using the following equation:

$$A_{serum}$$
 [Triglycerides] = $A_{standard}$ * r

$$n = 2.28 \rightarrow mmol/l$$
, $n = 200 \rightarrow mg/100 \, ml$, $n = 2 \rightarrow g/l$

Principle of the determination of cholesterol

rs will be hydrolyzed by cholesterol esterase. Cholesterol will be oxidized into cholest-4-en elesterol oxidase. H₂O₂ in the presence of phenol and amino-4- antipyrin forms a comp tion maximum between 505 nm.



Sample: Serum or plasma

solutions according to the table below:

1	2	3	4
1 ml	1 ml	1 ml	1 ml
230 landii 2	20 μΙ	280019 (15AC	140
2	<u> </u>	20 μl	124
-	126	12.47 (4) (1)	20 μl
20 μΙ	12月	120	100

and incubate them for 5 min at 37°C. Read the optical density of the samples (,

tion of cholesterol present in the samples can be calculated from the measured absorbance of the following equation:

$$A_{\text{serum}}$$
esterol, $A_{\text{total}} = A_{\text{standard}} * n$

nmol/l,
$$n = 200 \rightarrow mg/100 \text{ ml}$$
, $n = 2 \rightarrow g/l$

Determination of HDL (high density lipoprotein)

HDL fraction

will be precipitated in the presence of phosphotungstic acid-MgCl₂. After the centrifugation is supernatants will be determined according to the method described above for the tot

Reagents:

- 2 M MgCl₂ solution
- Precipitating reagent: 32 g/l phosphotungstic acid:0.4 mmol/l MgCl₂
- Plasma or serum of normal and 'ill' patients.

Separation procedude:

Add 500 µl plasma of normal and 'ill' patient, resp.,

50 μl precipitating reagent into marked Eppendorf tubes.

Mix the samples thoroughly and centrifuge them at 10 000 RPM for 15 min. After centrifugation the supernatant must be "clear". Pipette supernatants into test tubes and determine concentration of HDL-cholesterol.

Solutuons:

- Reagent#1: 50 mmol/l PIPES buffer pH=6.9; 24 mmol/l phenol
- Reagent#2: 250 U/I cholesterol-esterase; 250 U/I cholesterol-oxidase; 1000 U/I peroxidase; 0.5 mmol/I amino-4-antipyrin; 2.5 mmol/I NaCI
- Reagens#3: 1.3 mmol/l = 50 mg/dl (HDL-cholesterol standard)

<u>Serum samples:</u> (normal level of cholesterol, elevated level of cholesterol) pretreated with precipitating reagent (see separation of HDL fraction)

Measing LDL-Clevel

- ☐ LDL level calculated either: directly or by Friedwald equation
- ☐ LDL= Total cholesterol -(HDL+TG/5)
- □ vLDL=TG/5

Normal Range

LIPID PROFILE					
	DESIRABLE	BORDERLINE	HIGH RISK		
Cholesterol	<200	200-239	240		
	mg/dl	mg/dl	mg/dl		
Triglycerides	<150	150-199	200-499		
	mg/dl	mg/dl	mg/dl		
HDL	60	35-45	<35		
cholesterol	mg/dl	mg/dl	mg/dl		
LDL	60-130	130-159	160-189		
cholesterol	mg/dl	mg/dl	mg/dl		
Cholesterol/ HDL ratio	4.0	5.0	6.0		

Units converter

Cholestrol

mmol/l * 38.6 = mg/dl

TG

mmol/l * 88.5 = mg/dl

HDL and **LDL**

mmol/l * 40 = mg/dl

VLDL = TG/5

NOTES

- Measuring blood cholesterol level not need fasting? Cholesterol level is not affected by single meal but affected by long term pattern of eating (change from high fat diet to low fat diet for several weeks)
- Cholesterol level is <u>elevated</u> during pregnancy (6 weeks after delivery)
- Some drugs are known to increase cholesterol levels as anabolic steroids, beta blockers, epinephrine, oral contraceptives and vitamin D.

Thank you