
بسم الله الرحمن الرحیم

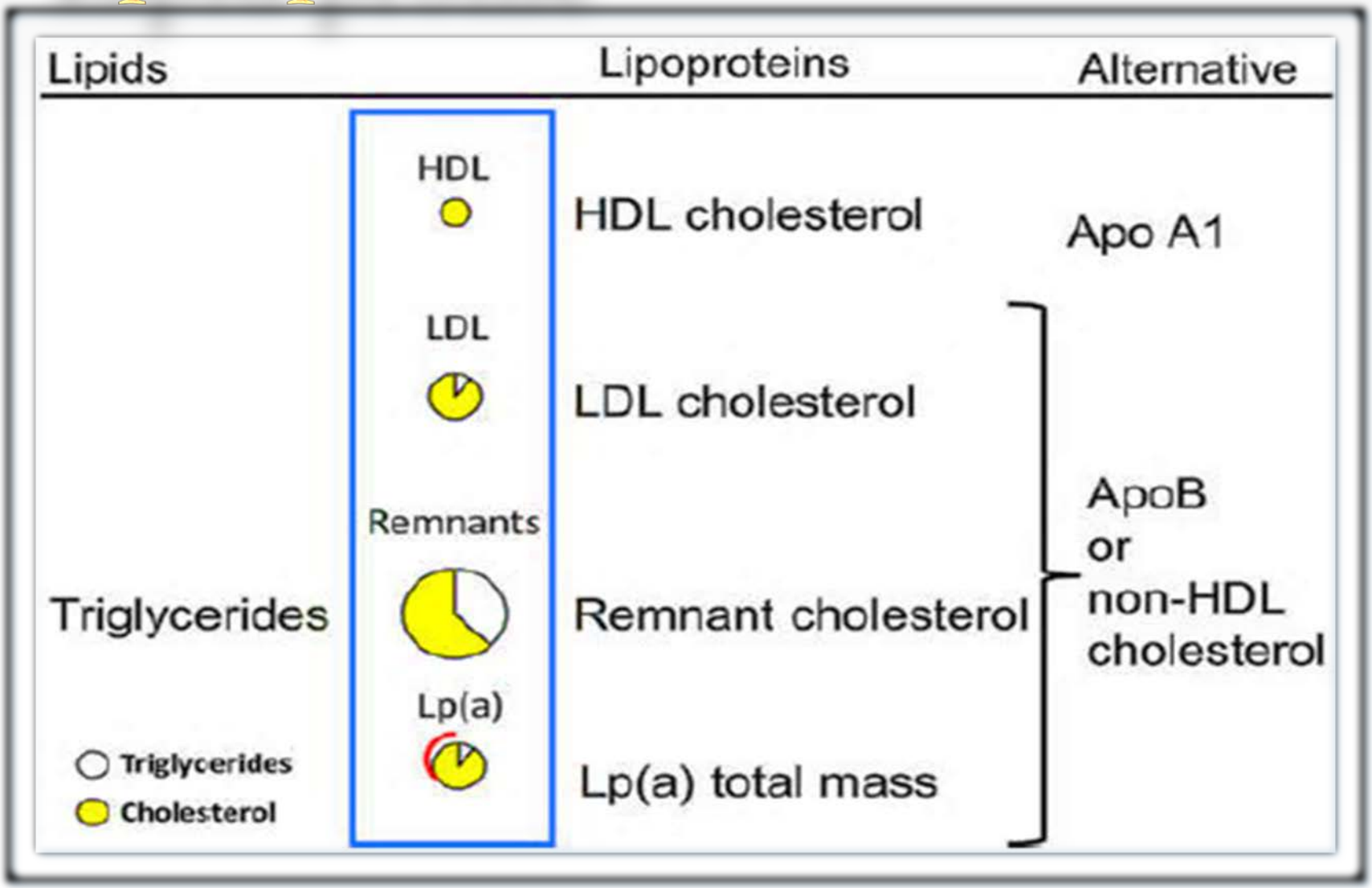
Measurement and inter pretation of lipid profile in the clinical laboratory

Cardiovascular disease (CVD) is major case of mortality and morbidity in the world

In the USA more than 600,000 die of the heart disease each years. of these with heart disease coronary is the most common kill in > 370,000 people annually.

the measurment of lipid panel is pivotal in the management of the patient at risk of CVD

Lipid profile



When to check lipid panel

- **Two different Recommendations**
  - **Adult Treatment Panel (ATP III) of the National cholesterol Education Program (NCEP)**
    - **Beginning at age 20: obtain a fasting (9 to 12 hour) serum lipid profile consisting of total cholesterol , LDL, HDL and tri glycerides**
    - **Repeat testing every 5 years for acceptable values**
  - **United States Preventative Services Task Force**
    - **Women age 45 years and older, and men ages 35 years and older undergo screening with a total and HDL cholesterol every 5 years.**
    - **If total cholesterol > 200 or HDL <40, then a fasting panel should be obtained**
    - **Cholesterol screening should begin at 20 years in patients with a history of multiple cardiovascular risk factors, diabetes , or family history of either elevated cholesteral levels or premature cardiovascular disease**

Preanalytical considerations

- It is important to standardize conditions under which blood specimen are drawn and prepared for analysis

  - Fasting or non-fasting
  - Biological variations:
  - Age
  - Sex
  - Pregnancy
  - Medical conditions: thyroid, liver, and kidney diseases , …
  - Acute illness: It is recommended that lipoproteins measurement should be made no sooner than 8weeks after any form of trauma or acute bacterial

Medication Induced Changes in Lipid and Lipoproteins

	LDL Cholesterol	Triglycerides	HDL Cholesterol
Cardiovascular /Endocrine			
Amiodarone	↑Variable	↔	↔
β-Blockers***	↔	↑10-40%	↓5-20%
Loop diuretics	↑5-10%	↑5-10%	↔
Thiazide diuretics (high dose)	↑5-10%	↑5-15%	↔
Sodium-glucose co-transporter 2 (SGLT2) inhibitors	↑3-8%	↔↓	↑Variable
Steroid Hormones/Anabolic Steroids			
Estrogen	↓7-20%	↑40%	↑5-20%
Select progestins	↑Variable	↓Variable	↓15-40%
Selective Estrogen Receptor Modulators	↓10-20%	↑0-30*	↔
Danazol	↑10-40%	↔	↓50%
Anabolic steroids	↑20%	↔	↓20-70%
Corticosteroids	↑Variable	↑Variable	↔

Antiviral Therapy			
Protease inhibitors	↑15-30%	↑15-200%	↔
Direct Acting Antivirals	↑12-27%	↔	↑14-20%
Immunosuppressants			
Cyclosporine and tacrolimus	↑0-50%	↑0-70%	↑0-90%
Corticosteroids	↑Variable	↑Variable	↔
Centrally Acting Medications			
First Generation antipsychotics	↔	↑22%	↓20%
Second Generation antipsychotics	↔	↑20-50%	↔
Anticonvulsants	↑Variable	↔	↑Variable
Other Medications			
Retinoids	↑15%	↑35-100%	↔**
Growth Hormone	↑10-25%	↔	↔↑7%

- **Posture**
- **Plasma vs serum**
- **Anticoagulants**
- **storage**

✓ Physiologic variation can be several time greater than analytic error

PHYSIOLOGIC VARIATION OF PLASMA LIPIDS, LIPOPROTEINS, AND APOLIPOPROTEINS

Component	CVP(%)*	CVP(%)**
Total cholesterol	5.0	6.4
Triglycerides	17.8	23.7
LDL-cholesterol	7.8l	8.2l
HDL-cholesterol	7.1	7.5
ApoA-l	7.1	_
ApoB	6.7	_

CVP,coefficient of physiologic variation; HDL, high-density lipoprotein; LDL, low density lipoprotein.

*Data from patients of lipid clinic(Kafonek et al., 1992)

**Data from the national cholesterol Education Program 1995 Working Group on Lipoprotein Measuremen

✓ measurement most be made in several blood samples. Taken at least week apart to establish the individuals Lipoprotein concentration usual

Fasting versus non-fasting

Before 2009 all societies guidelines and statements required fasting be for measuring a lipid profile for CVD risk prediction this was mainly du to.

1. **Increase seen in triglycerides during a fat tolerance test, and post prandial TG Remain in elevated for several hours.**
2. **Fried Wald equation used for calculation of LDL-C uses fasting TG value.**
3. **Most reference value for serum lipid are established on fasting blood specimen .**

- **Among all studies comparing non-fasting with fasting lipid profile showed lipid and lipoprotein only change minimally in response to normal food intake.**

Maximal mean changes were 26 mg/dl foe TG and 8 mg/dl for total cholesterol, 8 mg/dl for LDL, 4 mg/dl for HDL-C

- **in 108602 individual from the Copenhag general population Study in random non-fasting samples TG, TC, LDL-C, remnant, non HDL-C LP $_{(a)}$ and ApoB , were all associated with higher risk of both, ischemic heart disease and myocardial infarction , Finally lipid – lowering trials using non fasting blood samples for assessment of lipid levels found that reducing levels of non-fasting lipids reduced the risk of CVD**

Maximal mean changes in lipids and lipoproteins at 1-6 h after consumption of habitual meals

	Study population	Random non-fasting compared with fasting concentrations			
		Triglycerides	Total cholesterol	LDL cholesterol	HDL cholesterol
Mora *et al.* (2008)[4]	26 330 women from the Women's Health Study	↑ 0.2 mmol/L ↑ 18 mg/dL ↑ 16%	↓ 0.1 mmol/L ↓ 4 mg/dL ↓ 1%	↓ 0.2 mmol/L ↓ 8 mg/dL ↓ 5%	No change
Langsted *et al.* (2008)[3]	33 391 men and women from the Copenhagen General Population Study	↑ 0.3 mmol/L ↑ 26 mg/dL ↑ 21%	↓ 0.2 mmol/L[a] ↓ 8 mg/dL ↓ 4%	↓ 0.2 mmol/L[a] ↓ 8 mg/dL ↓ 6%	↓ 0.1 mmol/L ↓ 4 mg/dL ↓ 6%
Steiner *et al.* 2011[30]	12 744 children from the National Health and Nutrition Examination Survey	↑ 0.1 mmol/L ↑ 9 mg/dL ↑ 10%	↓ 0.1 mmol/L ↓ 4 mg/dL ↓ 2%	↓ 0.1 mmol/L ↓ 4 mg/dL ↓ 4%	No change
Langsted and Nordestgaard (2011)[9]	2270 men and women with diabetes from the Copenhagen General Population Study	↑ 0.2 mmol/L ↑ 18 mg/dL ↑ 11%	↓ 0.4 mmol/L[a] ↓ 15 mg/dL ↓ 8%	↓ 0.6 mmol/L[a] ↓ 23 mg/dL ↓ 25%[b]	No change
	56 164 men and women without diabetes from the Copenhagen General Population Study	↑ 0.2 mmol/L ↑ 18 mg/dL ↑ 14%	↓ 0.3 mmol/L[a] ↓ 12 mg/dL ↓ 5%	↓ 0.3 mmol/L[a] ↓ 12 mg/dL ↓ 9%	No change
Sidhu and Naugler (2012)[29]	209 180 men and women from Calgary Laboratory Services	↑ 0.3 mmol/L ↑ 26 mg/dL ↑ 21%	No change	↓ 0.1 mmol/L ↓ 4 mg/dL ↓ 4%	No change

Values in mmol/L were converted to mg/dL by multiplication with 38.6 for cholesterol and by 88 for triglycerides.

[a]No longer statistically significant after adjustment for reduction in plasma albumin concentrations; thus this drop in total and LDL cholesterol is due to fluid intake, not to food intake. In other words, as water intake is allowed during the up to 8 h fasting before lipid profile testing,[2] this reduction in total and LDL cholesterol will also occur for fasting lipid profiles.

[b]Langsted *et al.* observed a drop in LDL cholesterol of 0.6 mmol/L (23 mg/dL) at 1–3 h after a meal in diabetics, which could be of clinical significance,[33] particularly if this precluded initiation of statin therapy. However, such an LDL reduction may also occur for fasting lipid profiles with water intake allowed,[2] as the likely explanation for the LDL cholesterol drop is fluid intake and ensuing haemodilution.

Influence of food in take on the plasma lipid profile

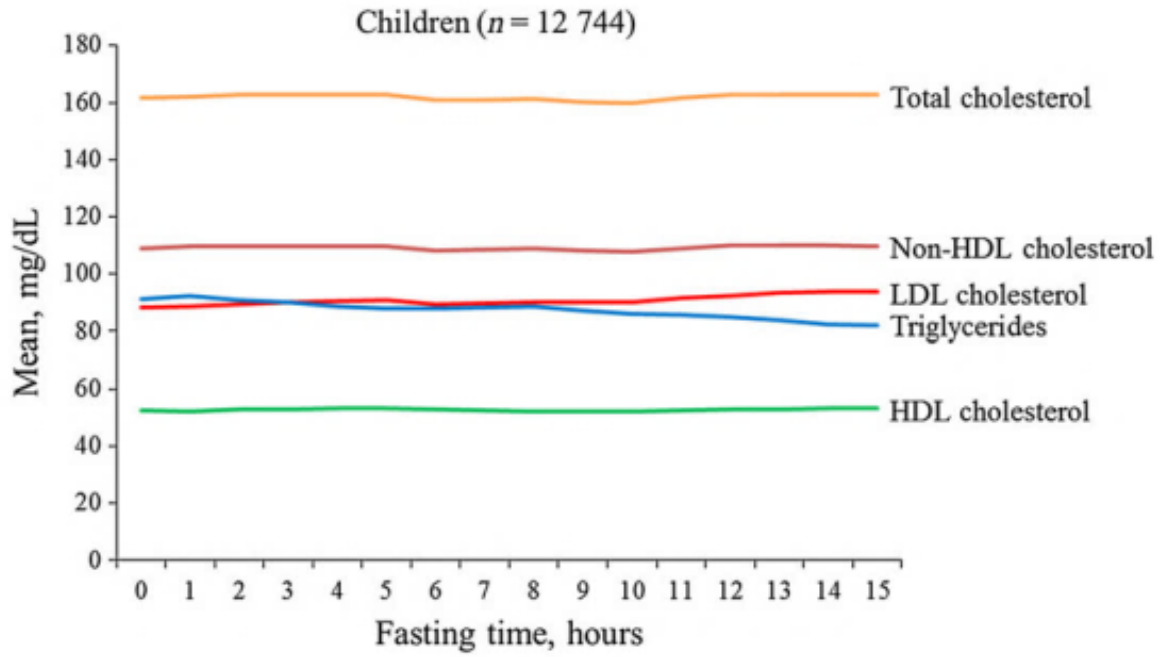


Figure 3 Mean concentrations of lipids and lipoproteins as a function of the fasting period following the last meal in children from the US general population. The last meal simply represents what the particular child chose to eat at that particular day before blood sampling, with no information or requirement on amount or type of food eaten. Based on 12 744 children from the National Health and Nutrition Examination Survey.[30]

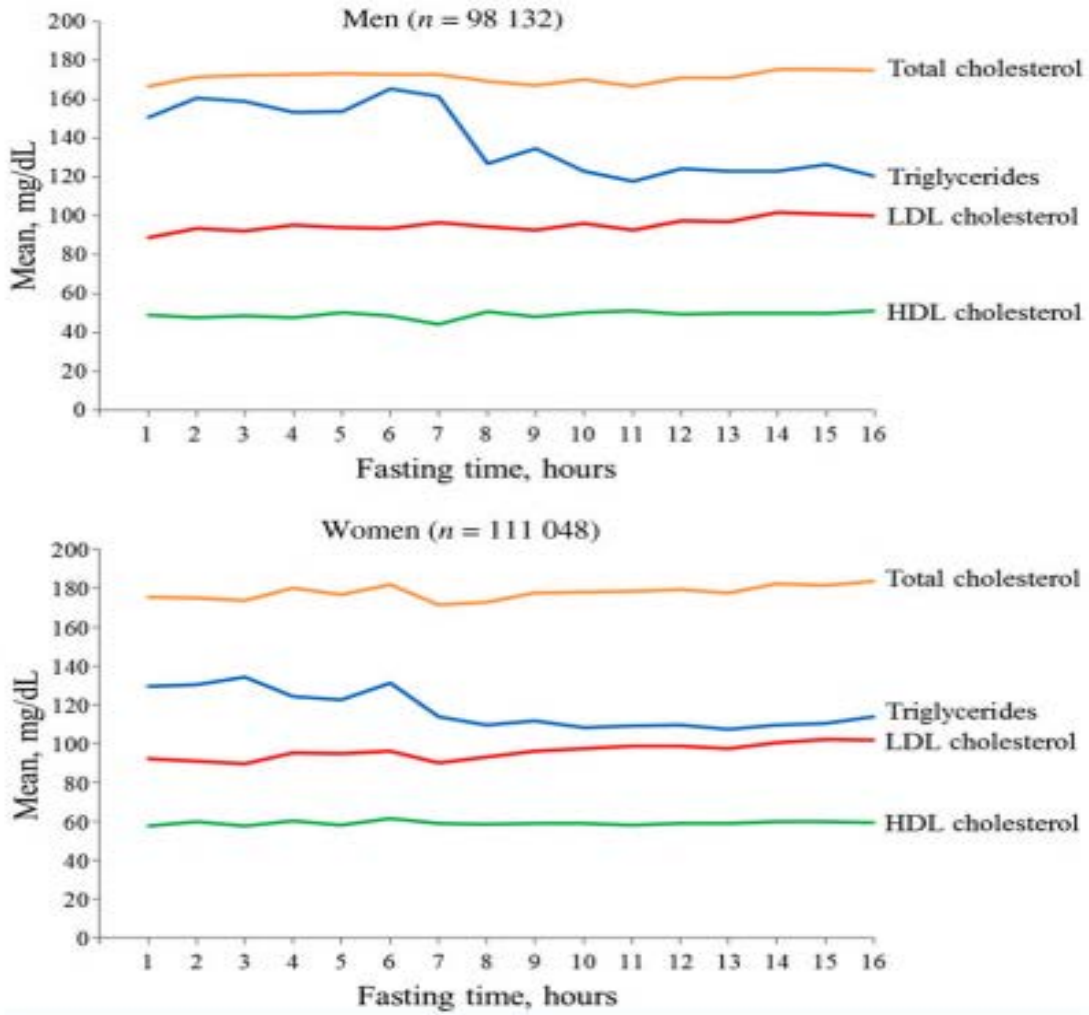


Figure 4 Mean concentrations of lipids and lipoproteins as a function of the period of fasting following the last meal in men and women from the Canadian general population. The last meal simply represents what the particular individual chose to eat at that particular day before blood sampling, with no information or requirement on amount or type of food eaten. Based on 209 180 men and women from Calgary Laboratory Services.[29]

Maximal mean change after habitual food intake

Copenhagen General Population Study

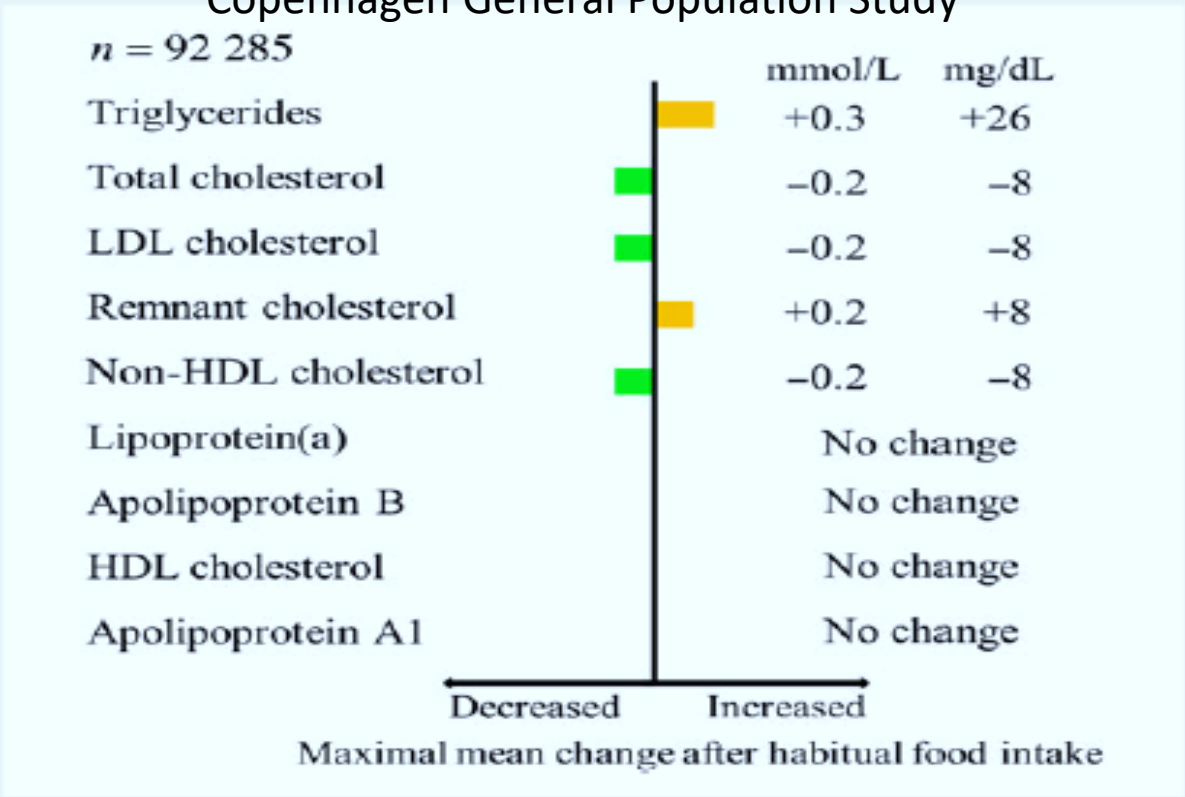


Figure 5 Maximal mean changes at 1–6 h after habitual food intake of lipids, lipoproteins, and apolipoproteins as part of standard and expanded lipid profiles in individuals in the Danish general population. Calculated remnant cholesterol is non-fasting total cholesterol minus low-density lipoprotein cholesterol minus high-density lipoprotein cholesterol. Calculated non-high-density lipoprotein cholesterol is total cholesterol minus high-density lipoprotein cholesterol. Adapted and updated from Langsted *et al.*,[3,34] based on 92 285 individuals from the Copenhagen General Population Study recruited in 2003 through 2014. Of all participants, 12% were receiving statins. Values in mmol/L were converted to mg/dL by multiplication with 38.6 for cholesterol and by 88 for triglycerides.

Table 4 When to use non-fasting and fasting blood sampling to assess the plasma lipid profile

	Patients for lipid profile testing
Non-fasting	In most patients, including: • Initial lipid profile testing in any patient • For cardiovascular risk assessment • Patients admitted with acute coronary syndrome[a] • In children • If preferred by the patient • In diabetic patients[b] (due to hypoglycaemic risk) • In the elderly • Patients on stable drug therapy
Fasting	Can sometimes be required if: • Non-fasting triglycerides >5 mmol/L (440 mg/dL) • Known hypertriglyceridaemia followed in lipid clinic • Recovering from hypertriglyceridaemic pancreatitis • Starting medications that cause severe hypertriglyceridaemia • Additional laboratory tests are requested that require fasting[c] or morning samples (e.g. fasting glucose[c], therapeutic drug monitoring)

[a]Will need repeated lipid profile testing later because acute coronary syndrome lowers lipid concentrations.

[b]Diabetic hypertriglyceridaemia may be masked by fasting.

[c]In many countries, fasting blood sampling is restricted to very few analytes besides lipid profiles: one example is fasting glucose; however, in many countries, even fasting glucose measurement is being replaced by measurement of haemoglobin A1c without the need to fast.

Abnormal plasma lipid, lipoprotein, and apolipoprotein concentration values that should be flagged in laboratory reports based on desirable concentration cut-points

Abnormal concentrations		
	Non-fasting (mg/dl)	Fasting (mg/dl)
Triglycerides	≥175	≥150
Total cholesterol	≥190	≥190
LDL cholesterol	≥115	≥115
Remnant cholesterol	≥35	≥30
Non-HDL cholesterol	≥150	≥145
Lipoprotein (a)	≥50	≥50
Apolipoprotein B	≥100	≥100
HDL cholesterol	≤40	≤40
Apolipoprotein A1	≤125	≤125

Reliability of measurement

TABLE 18.8

NCEP Guidelines for Acceptable Measurement Error

Analyte	Total Error	Bias	CV*
Cholesterol	≤9%	≤3%	≤3%
Triglyceride	≤15%	≤5%	≤5%
HDL-cholesterol	≤13%	≤5%	≤4%†
LDL-cholesterol	≤12%	≤4%	≤4%

HDL, High-density lipoprotein; *LDL,* low-density lipoprotein; *NCEP,* National Cholesterol Education Program.

*Coefficient of variation defined as standard deviation/mean × 100.

†Precision criteria applied to HDL-cholesterol levels of 42 mg/dL (1.09 mmol/L) and higher. At lower levels, CV is not used; rather, standard deviation should not exceed 1.7 mg/dL (0.044 mmol/L).

Analytical methodology

- Total cholesterol
- Triglycerides
- lipoproteins

Cholesterol Estimation

CHEMICAL METHODS:

- *Abell Kendall Method (Former Reference Method):*
  - Principle: 3 step
    - Cholesterol is hydrolyzed with alcoholic KOH
    - Unesterified cholesterol is extracted with petroleum jelly
    - Measured using the L-B Reaction
  - Liebermann-Burchardt Reaction (L-B Reaction):

    Cholesterol + Sulfuric acid + Acetic anhydride => bluish green solution
- Bloors Method:
  - Principle: 2 step
    - Cholesterol is extracted using an alcohol ether mixture
    - Measured using the L-B Reaction

ENZYMATIC METHOD:

- *Cholesterol Oxidase Method (Routine Lab – Assay of Choice):*
- Principle:

  Cholesterol ester + H_2O $\xrightarrow{\text{cholesterol esterase}}$ Free cholesterol

  Free Cholesterol $\xrightarrow{\text{cholesterol oxidase}}$ 4 cholestene-3-one + H_2O_2

  - Trinders Reaction:

    H_2O_2 + 4-aminophenazone $\xrightarrow{\text{peroxidase}}$ Quinoneimine dye (red) + H_2O

  - Read at 500nm wavelength
  - Linear up to 600 – 700mg/dL (15.54 – 18.13mmol/L)
- Advantages (in comparison to the Chemical Method):
  - Precise and accurate
  - Lesser interferences – bilirubin, ascorbic acid, Hb
  - Smaller sample quantity
  - Rapid; does not require preliminary extraction step
  - Can be used to measure unesterified cholesterol by omitting de-esterification step
  - Mild reagents; better suited for automated analysers

- Disadvantages:
  - They are not absolutely specific for cholesterol.
    - Cholesterol oxidase reacts with other sterols e.g plant sterol
  - Ascorbic acid and Bilirubin interfere by consuming H_2O_2
    - Bilirubin interference can produce falsely high or low values
    - Significant only at conc >5mg/dL decreasing Chol values by 5 – 15%

Classification of total cholesterol

< 200 mg/dl	Desirable
200 – 239 mg/dl	Borderline high
≥ 240 mg/dl	high

Familial hyper cholesterolmia

200-500 mg/dl	Heterozygote
600-1000 mg/dl	Homozygotes

Triglyceride measurement

CHEMICAL METHOD:

- First, Lipids are extracted using chloroform and phospholipids and removed by zeolite absorption
- *Van Handel and Zilversmith Method (former Reference Method):*
- Principle:
  - TAG $\xrightarrow{\text{alcoholic KOH}}$ -> Glycerol + Fatty acids
  - Glycerol + periodic acid ----------> Formaldehyde
  - Formaldehyde + Chromotropic acid----------> Blue solution

ENZYMATIC METHOD:

- *Glycerol Kinase Method:*
- Principle:
  - TAG + $3H_20$ $\xrightarrow{lipase}$ Glycerol + 3fatty acids
  - Glycerol + ATP $\xrightarrow{glycerol\ kinase}$ Glycerophosphate + ADP
  - Glycerophosphate $\xrightarrow{Glycerophosphate\ Oxidase}$ Dihydroxyacetone + H_2O_2

  Trinder's reaction:

  - H_2O_2 + Chromogen $\xrightarrow{peroxidase}$ Pink compound + H_2O
- Read absorbance at 500nm wavelength, and linear up to 700mg/dL
- Merits:
  - Fairly specific

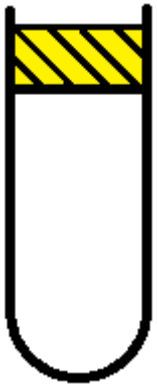
Triglyceride category

Less than 150mg/dl	Normal
150-199 mg/dl	Mildy high
≥ 500 mg/dl	Very high

- Triglyceride >880 mg/dl Chylomicronaemia syndrome with high risk of acute pancreatitis

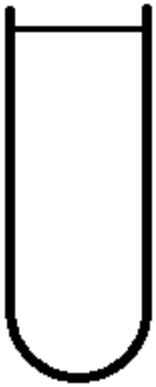
Lipoprotein measurement

Standing plasma test



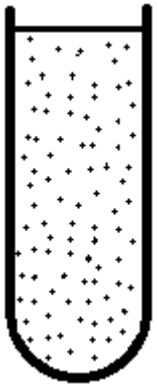
Creamy layer (chylomicrons) Over a clear layer

I



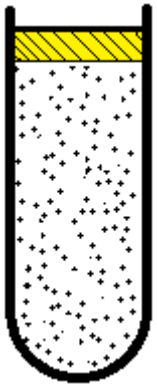
Usually clear

IIa



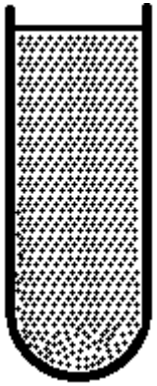
Clear or faintly turbid throughout no chylomicrons

IIb



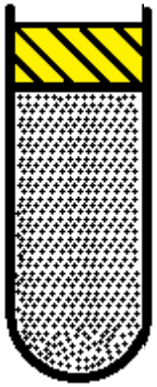
Turbid
A faint creamy layer may be Present at top

III



Turbid
No chylomicrons

IV

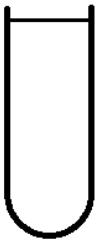


Creamy layer Over a turbid layer

V

Typical appearance of serum after standing 18 h at 4°C .

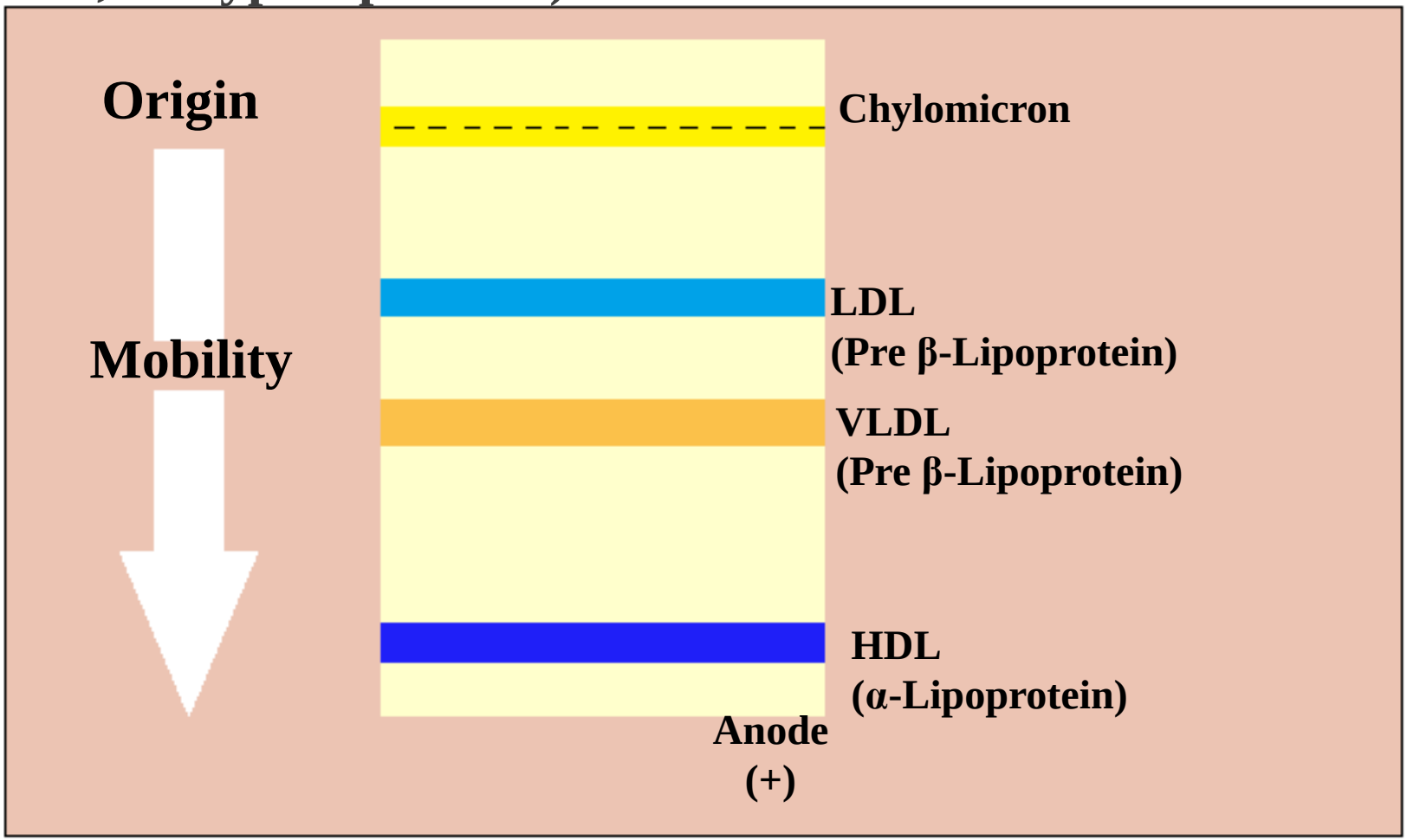
Creamy layer of chylomicrons.
Turbid layer associated with increased pre-β lipoprotein.



Normal Clear

Lipoprotein Electrophoresis

- Used to identify rare familial disorders(e.g Type I, III, V Hyperlipidemia)



- **Ultracentrifugation method**
  - **Analytical Ultracentrifugation**
  - **Preparative Ultracentrifugation :**

    Uses sequential density adjustment of serum fractionate major and minor classes of LP
- **Polyanion precipitation:**
  - **Lipoproteins are precipitated with polyanios (heparin sulfate, dextran sulfate and phosphotungstate)**
  - **Reaction should be in the presence of divalent cations Mg^{2+} , Ca^{2+} and Mn^{2+}**
  - **Most commonly for HDL assay**

- **β quantification (reference method)**
  - **Ultracentrifugation at density 1.006 kg/L to float and remove VLDL and any chylos measurement of TC in bottom fraction (LDL-C + HDL-C)**
    Precipitation on bottom fraction with heparin Mn^{2+} To remove LDL measurement of total cholesterol in supernatant with equal HDL-C
    But which is time consuming, expensive, and large specimen is need typically 5 ml, and not very feasible in the clinical practice

Indirect method Fried Wald equation

- ✓ **The relationship between serum LDL-C and CVD is well established**
- ✓ **Therefor accurate Estimation LDL-C is essential**
- ✓ **FF have been used extensively for estimation of LDL-C, LDL-C =TC - (HDL-C +TG (mg/dl)/5)**
- ✓ **It is cost effective and convenient**
- ✓ **FF fasting sample are required**
- ✓ **Some condition such as TG > 400 mg/dl and Disorder related to lipoprotein decreased the accuracy**

- **In addition FF* there are several other formula (FF) modification for estimation of LDL-C**
- **Martin-Hopknis calculation the overall accuracy of the Mar-hopkins equation compared with reference method was 92% as opposed to 85% for the FF**
- **Ghasemi et al**

modified Friedewald formulae for estimating low-density lipoprotein cholesterol according to triglyceride levels(Ghasemi et al)

TG concentration (mg/dl)	Modified Friedewald formulae
<100	M-LDLC=TC-HDLC-TG/2.7
100-200	M-LDLC=TC-HDLC-TG/3.7
200-300	M-LDLC=TC-HDLC-TG/4.6
300-400	M-LDLC=TC-HDLC-TG/5

Direct method

- **Direct assay recommended by the **NCEP working group measurement**
- **The homogenous or direct LDL-C and HDL-C assay have Largely replaced**
- **Homogenous direct LDL-C methods are useful when TG are elevated even at relatively high concentration (600 mg/dl)**
- **Direct assays have been adapted for use on variety of analyzer**
- **Direct method with acceptable accuracy and precision when compared with reference method**
- **In general they use a combination of two reagent. The first reagent usually selectively removes non LDL-C lipoproteins (and/or stabilizes or inhibits LDL from reacting with enzymes). The second reagent release cholesterol from LDL so that it can be**

Direct method for determination of LDL

1- Elimination of lipoprotein no-LDL

Cholesterol esters $\xrightarrow{CHE}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{CHOD}$ Cholestenone + H_2O_2

$2H_2O_2$ $\xrightarrow{Catalase}$ $2H_2O + O_2$

2-Measuremwnt of HDL-C

Cholesterol esters $\xrightarrow{CHE}$ CHE Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{CHOD}$ Cholestenone + H_2O_2

$2H_2O_2$ + HDAOS + 4-AA $\xrightarrow{POD}$ Quinone Pigment + $4H_2O$

CHE: cholesterol esters CHOD: Cholesterol oxidase

LDL-C	
< 100 mg/dl	Optimal
100 – 129 mg/dl	Near or above optimal
130 - 159 mg/dl	Borderline high
160 – 189 mg/dl	High

- LDL cholesterol > 500 mg/dl Homozygous familial hypercholesterolemia with extremely high cardiovascular risk
- LDL cholesterol > 190 mg/dl Heterozygous familial hypercholesterolemia with extremely high cardiovascular risk
- LDL cholesterol in children > 155 mg/dl Heterozygous familial hypercholesterolemia with extremely high cardiovascular risk

Direct method for determination of HDL

1-Elimination of lipoprotein no-HDL

Cholesterol esters $\xrightarrow{\text{CHE}}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{\text{CHOD}}$ Cholestenone + H_2O_2

$2H_2O_2$ $\xrightarrow{\text{Catalase}}$ $2H_2O_2 + O_2$

2-Measuremwnt of LDL-C

Cholesterol esters $\xrightarrow{\text{CHE}}$ CHE Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{\text{CHOD}}$ Cholestenone + H_2O_2

$2H_2O_2$ + TOOS + 4-AA $\xrightarrow{\text{POD}}$ Quinone Pigment + $4H_2O$

CHE: cholesterol esters CHOD: Cholesterol oxidase

HDL

At risk	Desirable
Men: Less than 40 mg/dl	60 mg/dl Or above
Women: Less than 50 mg/dl	60 mg/dl Or above

Oddly enough people who naturally have extremely high DL levels

Above 100 mg/dl – appear to be at the higher risk of heart disease this maybe cased by genetic factors

Comparison between F-LDL-C and Direct LDL-C (D-LDL-C)

Inconsistent results in different studies:

- ✓ **Reports of positive deviation (F-LDL-C higher than D-LDL-C)**
  - ✓ **Iran ~12 mg/dl & ~7 mg/dl (m 2 studies)**
  - ✓ **South Africa ~ 12 mg/dl**
  - ✓ **Japan~ 10mg/dl, in Iran**
  - ✓ **India~ 6**
  - ✓ **Brazil~ 4 mg/dl**
  - ✓ **In diabetic patients~ 22 mg/dl**

Report of negative deviation (F-LDL-C lower than D-LDL-C):

- ✓ **US: 22 mg/dl**
- ✓ **Serbia: ~10mg/dl**
- ✓ **In schizophrenic: ~7 mg/dl**

Overestimation or under estimation of LDL-C concentration could result in overt of patient.

Non HDL

- The other biomarker which is utilized in prediction cardiovascular disease (CVD) is non HDL-C
- This biomarker can be used in both fasting and non-fasting state regard less of the TG concentration
- Non HDLc provide a more comprehensive assessment in certain individual
- It is easy to calculate and does not require addition cost
  Non HDL=Tc – HDL-C
- As a treatment targets for cholesterol lowering drug therapy
- Non HDL levels are often elevated in people with type II diabetes even when LDL levels are within the norm range

- **As a result multiple studies show that obese people have elevated non HDL-C levels**
- **Infection and inflammation increased level up non HDL-C**
- **NCEP: recommended which in case triglycerides increase more than 200 mg/dl non HDL to be used instead of LDL-C**
- **What is the normal range of non HDL**

  - **Age 19 and younger less than <120 mg/dl**
  - **Men age 20 and older less than 120 mg/dl**
  - **Woman age 20 and older less than 130 mg/dl**


با سپاس
فراوان