Tikrit University

College of Nursing

Basic Nursing Sciences

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Bio Chemistry

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Triglycerides

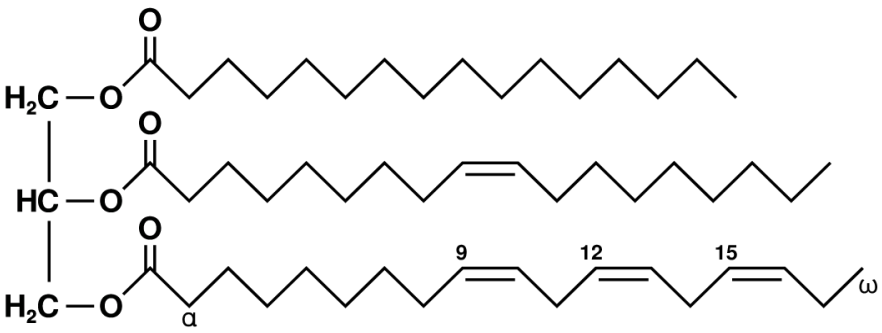
Triglycerides

Triglycerides are the fatty acid esters of glycerol and are the main component of fats in dietary fats and fat stores of animals. Cholesterol and triglycerides, being non-polar lipid (insoluble in water), must be transported in the plasma attached to different lipoprotein molecules. Triglycerides are our main source of energy and as they are so important we have two supplies

There are two sources of triglycerides:

1- An external source.supply from our diet

2- internal source made in the body by our liver.



structural formula of Triglycerides

The body obtains triglycerides from external sources, which is by eating foods that contain it On fats, as they travel through the lymph to the bloodstream and then to their storage sitesLipoproteins are lipoproteins that can be extracted from body tissues such as the liver and intestines and then go to other tissues for the purpose of storage or the process of demolition in order to obtain energy.

High triglycerides are often a sign of other conditions that increase the risk of heart disease and stroke, including obesity and metabolic syndrome - a group of conditions that include a lot of fat around the waist, high blood pressure, high triglycerides, and high levels of Blood sugar, abnormalities. Cholesterol levels.

triglycerides levels.

Season: cholesterol levels are slightly higher in cold weather. Higher levels were observed in winter and lower in spring and summer.

Food intake:

- Ingestion of fatty meals may cause elevated TG levels. Ingestion of monounsaturated fat reduces cholesterol.
- Plasma triglyceride concentration is reduced when sucrose intake is reduced. Eating

sugar and other carbohydrates raises triglycerides.

- Large protein meals at lunch or in the evening also increase the serum cholesterol for at least 1 hour after a meal. In vegetarian individuals, the LDL-cholesterol

VLDL-cholesterol are reduced by 37 % and 12%.

- Individuals who eat many small meals throughout the day tend to have concentration of LDL and HDL that are lower than when same type and amount of food is eaten in three meals.

Life-style : lipids are higher in sedentary and poor diet habits.

To determine lipid profile, the patients should be on their usual diet for 2 weeks and are neither gaining nor losing weight before the blood is taken. Patient should fast for at least 12 hours before sampling. Blood should be drawn after an overnight fast.

- Chylomicrons are cleared within 6 – 9 hours and their presence after 12 hours fast .is abnormal. Chylomicrons, being derived from dietary fat, should normally have been cleared; a pathological disturbance may thus be inferred if they are present.
- In health, in the fasting state, plasma is clear. Following a meal, it often becomes opalescent owing to the light-scattering properties of chylomicrons and VLDL. At triglyceride concentrations above about 4 mmol/L, the plasma

becomes increasingly turbid; with severe hypertriglyceridemia, it appears milky (lipemic). If plasma is left undisturbed, chylomicrons float to the surface, leaving a clear infranatant layer;

VLDL remain in suspension. The LDL do not scatter light and, even at high plasma cholesterol concentrations, the plasma remains clear.

Medical conditions: many drugs and diseases like thyroid, liver, and kidney diseases affect lipid profile.

Acute illness : It is recommended that lipoproteins measurement should be made no

sooner than 8 weeks after any form of trauma or acute bacterial/viral infection and 3 - 4 months after child birth. When lipid studies are done on a patient who has had a myocardial infarction or stroke, blood should either be taken within 24 hour or after an interval of three months, because the metabolism of lipoproteins is disturbed during the convalescent period and analytical results may be misleading. Positional variations occur, cholesterol levels are lower when sitting, versus standing and lower when recumbent verses sitting. Decreases of as much 10% in concentration of TC, LDL-C, HDL-C and apo-A-I and B, have been observed after

20 minutes recumbence.Prolonged venous occlusion leads to increase in cholesterol concentration by 10 - 15%. Ideally, the tourniquet should be in place no longer than one minute to prevent hemoconcentration which can cause falsely elevated results.

Mild exercise produces a slight decrease in concentration of cholesterol and TG that may persists for several days. Those who walk for about 4 hours each week have an average cholesterol concentration 5% lower and HDL-C concentration 3.4% higher than inactive persons.

Smoking: the plasma cholesterol , triglyceride and LDL cholesterol concentration are higher by about 3 %, 9.1 % and 1.7 % respectively in smokers than in non smokers.

The HDL cholesterol is lower in smokers than in non-smokers.
Alcohol ingestion: when moderate amount of alcohol is ingested for 1week, the serum TG concentration is increased by more than 20 mg/dL. Prolonged moderate ingestion of alcohol may increase HDL-C concentration. Alcohol should not have been taken on the evening before blood sampling. Alcohol is a common cause of hypertriglyceridemia even in patients who have otherwise fasted. Either plasma or serum can be used when TC, TG and HDL-C are measured. Serum can be used when it is necessary to store samples for weeks or months. It is generally recommended that plasma be stored in the liquid state when it is to be used for lipid and, particularly, lipoprotein analysis or for lipoprotein electrophoresis studies. The levels of all lipoproteins may decrease with storage.

Desirable value: < 150 mg/dL (< 1.7 mmol/L SI units)
Border line high value: 150 - 199 mg/dL (1.7 - 2.25 mmol/L SI units)
High value: 200 – 499 mg/dL (2.26 - 5.64 mmol/L SI units)
Specimen :
- Usually serum used in analysis, obtained from venous blood. The blood should be
rapidly centrifuged to minimize the spontaneous hydrolysis of triglycerides to glycerol and fatty acids in the blood. If the blood sample cannot be analyzed for triglycerides within 24 hours, freezing the samples at −20°C, preferably at −40°C to
−60°C, or colder (such as −80°C) is recommended, using tubes specifically designed
for low temperature storage.
- Fasting is required for 12-16 hours before testing. Only water is permitted also no
alcohol should be taken 24 hours before test. A fasting sample is essential for

triglyceride analysis, since triglyceride levels increase as soon as 2 hours postprandially and reach a maximum at 4 to 6 hours. Samples drawn from non1fasting patients are not suitable for analysis.

- Most current methods use chemical or enzymatic procedures to determine the glyceride glycerol concentration, which is then converted to the equivalent mass concentration of an average triglyceride.

Lipoproteins

Normal value of HDL : 40 - 60 mg/dL (1.04 - 1.55 mmol/L SI units)

Values of HDL < 60 mg/dL: Considered a negative risk factor of heart disease

Values of HDL > 40 mg/dL: Considered a major risk factor for heart disease

Optimal value of LDL : > 100 mg/dL (> 2.6 mmol/L SI units)

Near optimal value of LDL: 100- 129 mg/dL (2.6 - 3.35 mmol/L SI units)

Border line high value of LDL: 130 - 159 mg/dL (3.36 - 4.11 mmol/L SI units)

Normal VLDL level is between 2 and 30 mg/dL (0.1 to 1.7 mmol/L). Normal value ranges may vary slightly among different laboratories.

Specimen :

- Usually serum used in analysis, obtained from venous blood.
- Fasting is required for 12-14 hours before testing. Although non-fasting conditions

do not appear to influence the blood HDL-C levels in most people, the postprandial

lipemia has the potential of interfering with many of the analytical methods. To minimize this analytical problem, it is always good laboratory practice to request

fasting specimens. Only water is permitted also no alcohol should be taken 24 hours

before test.

- Serum or plasma can be used as the sample, but since plasma is usually preferred

for lipid analysis, the HDL analysis will most frequently be performed on the same
plasma specimen. The sample should be removed from the blood clot within 2 hours
and may be stored at 4°C for up to 2 days. If specimens are to be kept for longer than 48 hours, they should be frozen. The HDL is relatively labile, and freeze/thaw
cycles have been shown to affect some of the precipitation methods. However, if
specimens are frozen, they should be kept at temperatures below −50°C, at which
temperature they are stable for up to 2 years. Once thawed, specimens should be gently mixed.

Triglyceride, total cholesterol and HDL cholesterol concentrations can easily be measured in the laboratory. Most clinical laboratories utilize the convenient method
of Friedewald to estimate or calculate LDL cholesterol as following:

LDL = total cholesterol − (HDL +VLDL)

LDL mmol/L= total cholesterol – (HDL + TG/2.2)

This formula is invalid if the triglyceride concentration exceeds 4.5 mmol/L.

LDL mg/dL= total cholesterol – (HDL + TG/5)

This formula is invalid if the triglyceride concentration exceeds 400 mg/dL

Triglycerides level in serum

PRINCIPLE

Fossati and Prencipe method associated with Trinder reaction.

Reaction scheme is as follows:

Triglyceride ——Lipase—→ Glycerol + free fatty acids

Glycerol + ATP $\xleftrightarrow{}$ GK Glycerol 3 Phosphate + ADP

Glycerol 3 Phosphate + O2 $\xleftrightarrow{}$ GPO Dihydroxyacetone Phosphate + H2O2

H2O2 + 4-Chlorophenol + PAP $\xrightarrow{}$ POD Quinoneimine (pink)+ H2O

The absorbance of the coloured complex (quinoneimine), proportional to the amount of triglycerides in the specimen, is measured at 500 nm.

MANUAL PROCEDURE

Let stand reagent and specimens at room temperature.

Pipette into well identified test tubes	Blank	Standard	Assay
Reagent	1 mL	1 mL	1 mL
Demineralised water	10 µL		
Standard		10 µL	
Specimen			10 µL
Mix. Let stand for 5 minutes at 37°C or 10 minutes at room temperature. Record absorbance at 500 nm (480-520) against reagent blank. Reaction is stable for 1 hour.			

Note: Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$